Application of Lipase-Catalyzed Regioselective Esterification in the Preparation of Digitonin Derivatives

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The oligosaccharide chain of the monodesmosidic haemolytic saponin digitonin (**1**) undergoes an efficient and regioselective acylation in organic solvent by use of Novozym 435 (lipase B from *Candida antarctica* supported on acrylic resin) in the presence of an activated ester. With vinyl acetate, acetylation occurs at C-6 OH of glucose(II) and C-4 OH of xylose to afford the previously unreported diacetyl derivative **2** and the monoacetyl derivatives **3** and **4**. With vinyl laurate only the monolauryl derivative **5** is formed. The structures of these acylated digitonins have been established using modern 2D NMR techniques, which allowed complete assignments of all proton resonances. The hemolytic activity of derivatives **2**−**5** is significantly reduced compared to that of digitonin.

Digitonin (**1**), a complex monodesmosidic steroidal saponin isolated from the seeds of *Digitalis purpurea* L.[1], has the distinctive characteristic of causing the rupture of the membrane of red blood cells. This hemolytic activity is shared by other natural saponins, and it is greatly influenced by the structure of the aglycon and by the number and type of sugars, as well as by their glycosidic linkages. However, despite systematic studies, no defined structure−activity relationships are presently known.[2] For many saponins, especially for spirostanol glycosides, it is assumed that hemolysis depends on the ability to form a complex with cholesterol, a central component of the erythrocyte membrane.[3] A 1:1 complex of digitonin has been prepared by us, and extensive NMR studies have been undertaken to clarify the sites of interaction between these two molecules.[4] As a part of this work, we modified the digitonin molecule by preparing specific acylated derivatives in order to evaluate their hemolytic activity and measure their ability to form complexes with cholesterol. These data would furnish a better understanding of structural requirements for the hemolytic mechanism and provide information on the structure of the digitonin-cholesterol complex.

The digitonin molecule contains a branched pentasaccharide chain and therefore, due to the presence of multiple hydroxy functions of similar reactivity, represents a challenging target for selective modification. As for carbohydrates,[5] the chemical synthesis of individual acyl derivatives would require multistep protection−deprotection procedures and tedious chromatographic separations, resulting in low overall yields of the desired product.

We have shown that esters present at specific positions of the saccharide moiety of flavonoid glycosides[6] and of ginsenoside Rg_1[7] can be prepared by reaction with an activated ester under the catalysis of enzymes in anhydrous organic solvents. Therefore, we hoped that this enzyme-mediated regioselective acylation could also be successfully applied to digitonin. This was indeed the case, and in this paper we report that, of the several hydroxyl groups present in this molecule, only the C-4 OH of xylose and C-6 OH of glucose(II) where recognized by *Candida antarctica* lipase in *tert*-amyl alcohol solution. In the presence of vinyl acetate and vinyl laurate this enzyme catalyzed the exclusive formation of the diacetate **2**, of the monoacetates **3** and **4**, and of the monolaurate **5**, respectively, whose structures rely on a complete analysis of their NMR spectra.

Results and Discussion

Due to its polyhydroxylated nature, digitonin **(1)** is soluble in polar solvents and barely soluble in solvents of low polarity. We found that **1** is moderately soluble in *tert*-amyl alcohol containing a limited amount of DMF (13%), and therefore, according to the protocol used for the esterification of ginsenoside Rg_1[7], we first used Novozym 435 (*Candida antarctica* lipase B supported on an acrylic resin) and vinyl acetate to attempt the acylation of **1**. Under these conditions a 75% conversion of **1** was observed after 96 h to form three products, **2**, **3**, and **4**, in nearly a 1:2:1 ratio.[8]

The less polar product **(2)** was recognized as a di-*O*-acetyldigitonin on the basis of its LSIMS [$(M-H)^-$ ion at $m/z = 1311$] and of its proton spectrum, which showed, among other things, two singlets at δ 2.05 and 2.06 for the acetate groups, a downfield-shifted AB portion of an ABX system at δ 4.20 and 4.42 for the acylated oxymethylene protons of a glucose or galactose unit, and a downfield-shifted ddd at δ 4.73, presumably due to H-4 of the xylose unit on the basis of its coupling constants.

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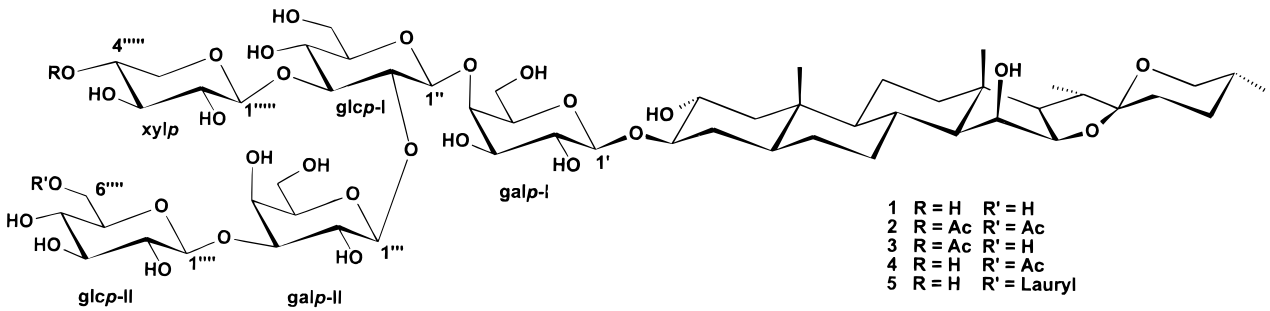

To unambiguously locate the positions of attachment of the acetyl residues to the oligosaccharide chain, a detailed NMR study was carried out. By means of a H−H COSY45 spectrum,[9] the strongly overlapped resonances of the five individual monosaccharide units (2 glc*p*, 2 gal*p*, and 1xyl*p*) and of the digitogenin aglycon were assigned. The sequence of protons in each sugar residue was determined by means of a two-dimensional total correlated spectroscopy (2D TOCSY) experiment[10] using a long mixing time (100 ms) to ensure propagation of the magnetization through the coupling networks. From these homonuclear-correlated experiments we deduced that the acetylated oxymethine at δ 4.73 belongs to the xylose unit whose H-1 resonates at δ 4.71 and that the acetylated oxymethylene protons at δ 4.20 and 4.42 are on the glucose mojety whose anomeric proton resonates at δ 4.55.

In the next step, the ^{13}C resonances were assigned using a HMQC experiment.[11] Information about the sequence of the oligosaccharide chain was obtained by observing the scalar coupling between an anomeric proton and the carbon atom of the linkage site at the neighboring residue by means of a HMBC experiment.[12] We found that 1-H of gal*p* I was connected to C-3 of digitonin, 1-H of glc*p* I to C-4 of gal*p* I, 1-H of xyl*p* to C-3 of glc*p* I, 1-H of gal*p* II to C-2 of glc*p* I, and finally, 1-H of glc*p* II to C-3 of gal*p* II. In this way, the anomeric proton at δ 4.55 was attributed to glc*p* II. In addition, there were long-range H−C couplings from the carbonyl atoms of the acetyl residues to H-4 (xyl*p*) and to the two downfield-shifted AB protons (glc*p* II), respectively. All these data are consistent with the strucutre of 6′′′′,4′′′′′-di-*O*-acetyldigitonin **2.** Our NMR measurements also showed that all the ^{1}H and ^{13}C chemical shift values of **2** were very similar to those previosly described for digitonin (**1**),[13] except those directly influenced by the functional changes at positions 6′′′′ and 4′′′′′.

On the basis of the above spectroscopic analysis, the structures of the two other reaction products **3** and **4** were determined in a straightforward manner. Both products showed pseudomolecular $[M - H]^-$ ions at *m*/*z* 1269 in the LSIMS spectrum, indicating the presence of a single acetyl residue in the molecule.

The product of medium polarity, **3** displayed the typical ddd of the acetylated H-4 of the xyl*p* moiety at δ 4.74. On the other hand, the most polar compound **4** showed ^{1}H NMR signals at δ 4.18 and 4.42 due to the AB portion of the ABX system of glc*p* II acetylated at C-6 OH. Thus, the two monoacetylated products are 4′′′′′-*O*-acetyldigitonin (**3**), and 6′′′′-*O*-acetyldigitonin (**4**), respectively. In both compounds, data for the proton resonances of the nonacylated portion of the molecule fit well with those of nonacetylated digitonin.

The hemolytic activity of digitonin and the mono- and di-acetylderivates was measured by determining the Hemolytic Index (HI), following the protocol described in the Österreichischen Arzneibuch.[14] The obtained values (for **1**, HI = 135 000; **2**, HI = 43 000; **3**, HI = 52 000; **4**, HI = 40 000) indicate that a small change in the outer shell of the polysaccharide chain leads to a significant decrease of the hemolytic activity, this effect being more noticeable when the primary OH of glc*p* II is acetylated. This portion of the molecule should, therefore, play an important role in the complex formation with cholesterol. In fact, we have not been able to prepare stable complexes between cholesterol and the three derivatives despite many attempts.

To strenghten the importance of this finding, we introduced a long fatty-acid chain into the digitonin molecule. When reacted with vinyl laurate under the same conditions as described previously, digitonin underwent moderate conversion into a single product, which proved to be the 6′′′′-*O*-lauryldigitonin (**5**) by the $[M - H]^-$ ion at *m*/*z* 1409 in the LSIMS spectrum and by the significant downfield shift of the two protons at C-6 of the glc*p* II moiety at δ 4.22 and 4.42.

The new derivative **5** can be considered an inverse bisdesmosidic saponin, in which the hydrophilic saccharide chain is connected to two different lipophilic aglycons. Bisdesmosidic saponins are known to possess a very low hemolytic activity, and this is the case with respect to **5**, which has the lowest activity (HI = 14 600) of all the derivatives **2**−**5** prepared by us.

In conclusion, we have shown that Novozym 435 is an efficient catalyst for the regioselective esterification of a saponin, which contains a complex branched pentasaccharide chain. This finding adds synthetic value to the use of this lipase for functional modification of polyhydroxy compounds, which, up to now, has been limited to glycosides containing single saccharide moieties.[6,7] It is not surprising that enzymatic acylation takes place on the 'external' sugars, since they are sterically more accessible to the enzyme active site. However, it is worth noting that the C-4 OH of the xylose moiety was selectively acylated in the presence of other more reactive primary OHs and of other secondary OHs with similar chemical reactivity.

The low potency of the digitonin derivatives **2**−**5** provides new insight into the structural requirements necessary for hemolytic activity. Future work will focus on modification of the sugar portion of **1** with hydrophilic substituents, for instance, the introduction of additional carbohydrate units by catalysis of glycosyltransferases.[15]

Experimental Section

General Experimental Procedures. Novozym 435 (Lipase B from *Candida antarctica* immobilized on an acrilyc resin) was a generous gift from Novo-Nordisk. Enzymatic transesterification of **1** was followed by TLC with precoated Si gel 60 F_{254} plates (Merck), eluent $CHCl_3$−MeOH−H_2O 8:5:0.5. Spots were visualized by spraying with anisaldehyde−H_2SO_4 reagent (Komarowsky's reagent) followed by heating. Melting points were determined on a Kofler melting point microscope. ^{1}H and ^{13}C NMR spectra at 500 and 125 MHz,

respectively, were recorded in MeOH-d_4 at 21 °C on a Bruker Avance 500 instrument. A 5-mm reverse probe head was used. MeOH signal was used as internal standard (δ ^{1}H: 3.3, δ ^{13}C: 49.0). H−H COSY: 45° mixing pulse. TOCSY: phase sensitive using TPPI, mixing time 100 ms (100 MLEV-17 cycles plus two trim pulses of 2.5 ms each). HMQC: phase sensitive mode using TPPI, BIRD sequence, GARP decoupled. HMBC: phase sensitive using TPPI, delay tuned to long-range couplings, 71 ms. Typical parameters for a 2D NMR experiment (COSY) were SI = 2K, 512 experiments, 32 scans per t_1 increment, 8 dummy scans, aquisition time 0.8 s, relaxation delay 1 s, zero filling to 4 K × 1 K, and cosine−square filter in both directons. LSIMS were obtained with a MAT 8500 (Finnigan) instrument (4.5 kV Cs beam, negative ion mode, glycerol as matrix).

Enzymatic Esterification of Digitonin (1) with Vinyl Acetate. Compound **1** (30 mg, isolated and purified as previously described[16]) was dissolved in 4 mL of *tert*-amyl alcohol and 0.6 mL of DMF, vinyl acetate (1.5 mL) and Novozym 435 (500 mg) were added, and the suspension shaken at 45 °C for 96 h. The enzyme was filtered off, the solvent evaporated, and the crude residue purified by flash chromatography.

6‴″,4‴″-di-*O*-Acetyldigitonin (2): obtained by flash chromatography using the eluent $CHCl_3$−MeOH−H_2O 8:2.5:0.2, 6 mg (18.7% yield); mp 175−177 °C; R_f 0.60; ^{1}H NMR δ 4.38 (1H, d, J = 7.9, H-1′), 3.83 (1H, dd, J = 9.6, 7.9 Hz, H-2′), 3.52 (1H, dd, J = 9.6, 3.4 Hz, H-3′), 4.03 (1H, dd, J = 3.9, 3.4 Hz, H-4′), 3.55 (1H, ddd, J = 7.6, 4.0, 3.9 Hz, H-5′), 3.65 (1H, dd, J = 11.7, 4.0 Hz, H-6a′), 3.85 (1H, dd, J = 11.7, 7.6 Hz, H-6b′), 4.62 (1H, d, J = 7.6 Hz, H-1″), 3.81 (1H, dd, J = 8.8, 7.6 Hz, H-2″), 3.76 (1H, dd, J = 8.8, 8.3 Hz, H-3″), 3.31 (1H, dd, J = 10.1, 8.3 Hz, H-4″), 3.33 (1H, ddd, 10.1, 2.4, 2.0 Hz, H-5″), 3.59 (1H, dd, J = 11.6, 2.0 Hz, H-6a″), 3.84 (1H, dd, J = 11.6, 2.4 Hz, H-6b″), 4.98 (1H, d, J = 7.5 Hz, H-1‴), 3.76 (1H, dd,, J = 9.1, 7.5 Hz, H-2‴), 3.62 (1H, dd, J = 9.1, 3.1 Hz, H-3‴), 4.08 (1H, dd, J = 3.7, 3.1 Hz, H-4‴), 3.59 (1H, ddd, J = 7.7, 4.1, 3.7 Hz, H-5‴), 3.68 (1H, dd, J = 11.8, 4.1 Hz, H-6a‴), 4.01 (1H, dd, J = 11.8, 7.7 Hz, H-6b‴), 4.55 (1H, d, J = 7.7 Hz, H-1⁗), 3.31 (1H, dd, J = 9.2, 7.7 Hz, H-2⁗), 3.38 (1H, dd, J = 9.2, 7.8 Hz, H-3⁗), 3.30 (1H, dd, J = 9.8, 7.8, Hz, H-4⁗), 3.51 (1H, ddd, J = 9.8, 3.2, 1.8, H-5⁗), 4.20 (1H, dd, J = 11.4, 1.8, H-6a⁗), 4.42 (1H, dd, J = 11.4, 3.2 Hz, H-6b⁗), 4.71 (1H, d, J = 7.8, Hz H-1⁗′), 3.34 (1H, dd, J = 9.3, 7.8 Hz H-2⁗′), 3.57 (1H, dd, J = 9.3, 8.1 Hz, H-3⁗′), 4.73 (1H, ddd, J = 10.0, 8.1, 5.4 Hz, H-4⁗′), 3.27 (1H, dd, J = 11.1, 5.4 Hz, H-5a⁗′), 4.02 (1H, dd, J = 11.1, 10.0 Hz, H-5b⁗′), 2.06 and 2.08 (s, each 3H, CH_3CO_2); 1.98 (1H, H-1α), 0.93 (1H, H-1β), 3.64 (1H, H-2β), 3.46 (1H, H-3α), 1.74 (1H, H-4α), 1.41 (1H, H-4β), 1.21 (1H, H-5α), 1.37 (1H, H-6α), 1.30 (1H, H-6β), 1.00 (1H, H-7α), 1.97 (1H, H-7β), 1.93 (1H, H-8β), 0.80 (1H, H-9α), 1.55 (1H, H-11α), 1.41 (1H, H-11β), 1.22 (1H, H-12α), 1.67 (1H, H-12β), 1.90 (1H, H-14α), 4.29 (1H, H-15α), 4.11 (1H, H-16α), 1.05 (1H, H-17α), 1.02 (3H, C(18)H_3), 0.90 (3H, C(19)H_3), 1.92 (1H, H-20α), 0.94 (3H, C(21)H_3), 1.63 (1H, H-23α), 1.49 (1H, H-23β), 1.91 (1H, H-24α), 1.70 (1H, H-24β), 1.60 (1H, H-25β), 3.46 (1H, H-26α), 3.36 (1H, H-26β), 0.79 (3H, C(27)H_3); ^{13}C NMR δ 102.8 (d, C-1′), 72.6 (d, C-2′), 75.6 (d, C-3′), 80.0 (d, C-4′), 75.4 (d, C-5′), 61.4 (t, C-6′), 104.6(d, C-1″), 80.6 (d, C-2″), 87.0 (d, C-3″), 70.5 (d, C-4″), 77.6 (d, C-5″), 63.1 (t, C-6″), 104.1 (d, C-1‴), 72.1 (d, C-2‴), 84.5 (d, C-3‴), 69.9 (d, C-4‴), 76.8 (d, C-5‴), 62.6 (t, C-6‴), 105.5 (d, C-1⁗), 75.2 (d, C-2⁗), 77.6 (d, C-3⁗), 71.5 (d, C-4⁗), 75.7 (d, C- 5⁗), 64.7 (t, C- 6⁗), 104.7 (d, C-1⁗′), 75.3 (d, C-2⁗′), 75.5 (d, C-3⁗′) 72.9 (d, C-4⁗′),-63.8 (t, C-5⁗′), 45.7 (t, C-1), 71.5 (d, C-2), 84.9 (d, C-3), 34.1 (t, C-4), 46.0 (d, C-5), 29.0 (t, C-6), 32.5 (t, C-7), 43.7 (d, C-8), 56.1 (d, C-9), 38.1 (s, C-10), 22.6 (t, C-11), 43.6 (t, C-12), 41.8 (s, C-13), 62.9 (d, C-14), 83.9 (d, C-15), 71.1 (d, C-16), 61.3 (d, C-17), 19.4 (q, C-18), 13.7 (q, C-19), 31.9 (d, C-20), 14.5 (q, C-21), 111.3 (s, C-22), 29.2 (t, C-23), 31.9 (t, C-24), 31.5 (d, C-25), 68.2 (t, C-26), 17.5 (q, C-27), 20.7 (q, *C*H_3COO), 20.8 (q, *C*H_3COO), 170.0 (s, CH_3*C*OO), 169.0 (s, CH_3*C*OO). LSIMS *m*/*z* 1311 [M − H]$^-$, 1269, 1252, 1209, 1137, 1107, 1065, 945, 933, 903, 885, 813, 771, 609.

4⁗′-*O*-Acetyldigitonin (3): obtained by flash chromatography using the eluent $CHCl_3$−MeOH−H_2O 8:3:0.25, 11.5 mg (37% yield); mp 210−213 °C; R_f 0.45; ^{1}H NMR δ 4.38 (1H, d, H-1′), 3. 83 (1H, dd, H-2′) 3.52 (1H, dd, H-3′), 4.03 (1H, dd, H-4′), 3.55 (1H, ddd, H-5′), 3.65 (1H, dd, H-6a′), 3.84 (1H, dd, H-6b′), 4.62 (1H, d, H-1″), 3.81 (1H, dd, H-2″), 3.76 (1H, dd, H-3″), 3.30 (1H, dd, H-4″), 3.34 (1H, ddd, H-5″), 3.58 (1H, dd, H-6a″), 3.89 (1H, dd, H-6b″), 4.98 (1H, d, H-1‴), 3.77 (1H, dd, H-2‴), 3.67 (1H, dd, H-3‴), 4.11 (1H, dd, H-4‴), 3.60 (1H, ddd, H-5‴), 3.71 (1H, dd, H-6a‴), 4.02 (1H, dd, H-6b‴), 4.56 (1H, d, H-1⁗), 3.30 (1H, dd, H-2⁗), 3.37 (1H, dd, H-3⁗), 3.47 (1H, dd, H-4⁗), 3.29 (1H, ddd, H-5⁗), 3.68 (1H, dd, H-6a⁗), 3.85 (1H, dd, H-6b⁗), 4.71 (1H, d, H-1⁗′), 3.34 (1H, dd, H-2⁗′), 3.58 (1H, dd, H-3⁗′), 4.74 (1H, ddd, H-4⁗′), 3.30 (1H, dd, H-5a⁗′), 4.02 (1H, dd, H-5b⁗′), 2.06 (3H, CH_3CO_2); LSIMS *m*/*z* 1269 [M − H]$^-$, 1227, 1209, 1107, 1095, 1065, 1047, 945, 933, 903, 885, 771, 609.

6⁗′-*O*-Acetyldigitonin (4): obtained by flash chromatography using the eluent $CHCl_3$−MeOH−H_2O 8:4:0.3, 6 mg (19% yield); mp 230−231 °C; R_f 0.31; ^{1}H NMR δ 4.38 (1H, d, H-1′), 3.83 (1H, dd, H-2′) 3.51 (1H, dd, H-3′), 4.03 (1H, dd, H-4′), 3.55 (1H, ddd, H-5′), 3.66 (1H, dd, H-6a′), 3.85 (1H, dd, H-6b′), 4.62 (1H, d, H-1″), 3.80 (1H, dd, H-2″), 3.74 (1H, dd, H-3″), 3.27 (1H, dd, H-4″), 3.33 (1H, ddd, H-5″), 3.58 (1H, dd, H-6a″), 3.89 (1H, dd, H-6b″), 4.98 (1H, d, H-1‴), 3.76 (1H, dd, H-2‴), 3.62 (1H, dd, H-3‴), 4.08 (1H, dd, H-4‴), 3.59 (1H, ddd, H-5‴), 3.68 (1H, dd, H-6a‴), 4.01 (1H, dd, H-6b‴), 4.54 (1H, d, H-1⁗), 3.25 (1H, dd, H-2⁗), 3.37 (1H, dd, H-3⁗), 3.24 (1H, dd, H-4⁗), 3.51 (1H, ddd, H-5⁗), 4.18 (1H, dd, H-6a⁗), 4.42 (1H, dd, H-6b⁗), 4.62 (1H, d, H-1⁗′), 3.24 (1H, dd, H-2⁗′), 3.90 (1H, dd, H-3⁗′), 4.51 (1H, ddd, H-4⁗′), 3.28 (1H, dd, H-5a⁗′), 3.85 (1H, dd, H-5b⁗′), 2.07 (3H, CH_3CO_2); LSIMS *m*/*z* 1269 [M − H]$^-$, 1227, 1209, 1137, 1095, 1065, 946, 933, 903, 771, 609.

Enzymatic Esterification of Digitonin (1) with Vinyl Laurate. Compound **1** (56 mg) was dissolved in 7 mL of *tert*-amyl alcohol and 1 mL of DMF, vinyl laurate (2 mL) and Novozym 435 (720 mg) were added, and the suspension shaken at 45 °C for 14 days. After filtration of the enzyme and solvent evaporation, the crude residue was purified by flash chromatography (eluent $CHCl_3$−MeOH−H_2O 8:2.5:0.1) to give 17 mg (26% yield) of 6⁗′-*O*-lauryldigitonin (**5**) and 26 mg of unreacted digitonin.

6⁗′-*O*-lauryldigitonin (5): mp 240−241 °C, R_f 0.42; ^{1}H NMR δ 4.42 (1H, d, H-1′), 3.88 (1H, dd, H-2′) 3.55 (1H, dd, H-3′), 4.07 (1H, dd, H-4′), 3.60 (1H, ddd, H-5′), 3.69 (1H, dd, H-6a′), 3.87 (1H, dd, H-6b′), 4.67 (1H, d, H-1″), 3.83 (1H, dd, H-2″), 3.79 (1H, dd, H-3″), 3.32 (1H, dd, H-4″), 3.38 (1H, ddd, H-5″), 3.62 (1H, dd, H-6a″), 3.93 (1H, dd, H-6b″), 5.00 (1H, d, H-1‴), 3.78 (1H, dd, H-2‴), 3.64 (1H, dd, H-3‴), 4.10 (1H, dd, H-4‴), 3.63 (1H, ddd, H-5‴), 3.70 (1H, dd, H-6a‴), 4.07 (1H, dd, H-6b‴), 4.57 (1H, d, H-1⁗), 3.35 (1H, dd, H-2⁗), 3.42 (1H, dd, H-3⁗), 3.33 (1H, dd, H-4⁗), 3.54 (1H, ddd, H-5⁗), 4.22 (1H, dd, H-6a⁗), 4.50 (1H, dd, H-6b⁗), 4.65 (1H, d, H-1⁗′), 3.27 (1H, dd, H-2⁗′), 3.55 (1H, dd, H-3⁗′), 3.94 (1H, ddd, H-4⁗′), 3.31 (1H, dd, H-5a⁗′), 3.70 (1H, dd, H-5b⁗′), 0.9−2.4 [23H, $CH_3(CH_2)_{10}CO_2$]; ^{13}C NMR δ 102.7 (d, C-1′), 72.5 (d, C-2′), 75.6[a] (d, C-3′), 79.9 (d, C-4′), 75.5[1] (d, C-5′), 61.4 (t, C-6′), 104.3 (d, C-1″), 80.8 (d, C-2″), 87.3 (d, C-3″), 70.5 (d, C-4″), 77.5 (d, C-5″), 63.1 (t, C-6″), 104.4 (d, C-1‴), 72.1 (d, C-2‴), 84.5 (d, C-3‴), 70.1 (d, C-4‴), 76.7 (d, C-5‴), 62.7 (t, C-6‴), 105.5 (d, C-1⁗), 75.2[a] (d, C-2⁗), 77.5 (d, C-3⁗), 71.0 (d, C-4⁗), 75.6[a] (d, C- 5⁗), 64.5 (t, C- 6⁗), 104.9 (d, C-1⁗′), 75.3[a] (d, C-2⁗′), 71.0 (d, C-3⁗′), 71.0 (d,C-4⁗′), 67.2 (t, C-5⁗′), 45.6 (t, C-1), 71.6 (d, C-2), 84.7 (d, C-3), 34.0 (t, C-4), 45.9 (d, C-5), 29.1 (t, C-6), 32.5 (t, C-7), 43.7 (d, C-8), 56.1 (d, C-9), 38.1 (s, C-10), 22.6 (t, C-11), 43.6 (t, C-12), 41.7 (s, C-13), 62.9 (d, C-14), 83.9 (d, C-15), 71.5 (d, C-16), 61.3 (d, C-17), 19.4 (q, C-18), 13.8 (q, C-19), 31.9 (d, C-20), 14.6 (q, C-21), 111.2 (s, C-22), 29.9 (t, C-23), 32.0 (t, C-24), 31.5 (d, C-25), 68.1 (t, C-26), 17.5 (q, C-27), 14.5 (q, *C*H_3−CH_2), 23.8 (t, CH_3−*C*H_2−CH_2), 26.1 (t, CH_2−*C*H_2−COOH), 30.3, 30.6 (2), 30.7, 30.8, 30.9 (t, $(CH_2)_6$), 33.1 (t, CH_3−CH_2−*C*H_2), 35.0 (*C*H_2−COOH), 175.4 (s, *C*OOH); LSIMS *m*/*z* 1409 [M − H]$^-$, 1277, 1227, 1211, 1065, 903, 771.

[a]Exchangeable signals.

Determination of the Haemolytic Index (HI). The Hemolytic Index of **1**−**5** were determined according to ÖAB 91,[14] using bovine blood suspension and a solution of Austrian Saponinstandard (0.01% saponinum album in phosphate buffer pH 7.4) as reference.

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