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Functional group-mediated biotransformation by *Curvularia lunata* NRRL 2178: synthesis of 3-dehydro-2-deoxy-ecdysteroids from the 3-hydroxy-2-mesyloxy analogues

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Abstract

Microbial transformation of ecdysteroids with 3-hydroxy-2-mesyloxy functional group by the fungus *Curvularia lunata* NRRL 2178 furnished 3-dehydro-2-deoxy analogues. The metabolites included 3-dehydro-2-deoxy analogues of 20-hydroxyecdysone, pterosterone, ponasterone A, 20-hydroxyecdysone 20,22-acetonide, shidasterone, and poststerone. The mild biotransformation conditions prevented the metabolites from C-5 epimerization.

Keywords: Ecdysteroid; Biotransformation; 3-Dehydro-2-deoxy-ecdysteroids; *Curvularia lunata* NRRL 2178

1. Introduction

Microbial transformation is the procedure most often employed in regio- and stereoselective synthesis of a compound with a special functionality, which is difficult to carry out by chemical means.[1] The main problem for microbial transformation is to find an appropriate microorganism to react with a certain substrate and the ability of the enzyme to react at the specific position to yield the required product. It is obvious that many strains of bacteria or fungi have to be screened for their capabilities to transform each substrate.[2,3] In most cases microorganisms of different species produce different metabolites.[4–6] Utilization of microorganisms with the same species but with different strains might lead to different metabolites. For example, four *Aspergillus niger* strains (one wild and three UV mutants) biotransformed flavonoids into different metabolites.[7] External factors have also been used to exert some effects on the reaction pathways. For example, changes of the growth media and culture conditions resulted in the bioconversion to different metabolites.[8] The biocatalytic ability of the fungus *Beauveria bassiana* to metabolize androst-4-en-3,17-dione was modified by controlling the pH of the media and this resulted in the production of different analogues of the steroid hormone.[9] Addition of some chemicals could alter reaction modes of biotransformation. For example, the biotransformation of taxoids by the fungus *Absidia coerulea* IFO 4011 in the presence of β-cyclodextrin gave a variety of metabolites.[10] The findings provided valuable information for the applications of microbial transformation in organic synthesis. However, prediction of the site of reaction of the substrate and the type of the newly created functionality of the metabolite is still with difficulty. An alternative approach of microbial transformation should therefore be proposed. It is of interest to investigate whether it is possible to prepare a substrate for a microorganism to react at a specific position and with a required mode of reaction.

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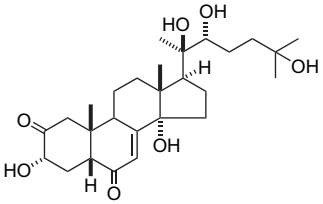
We have recently explored microbial transformation of ecdysteroids, the insect moulting hormones, for regio- and stereoselective synthesis of the rare 2-keto ecdysteroid, 2-dehydro-3-*epi*-20-hydroxyecdysone (**1**), from 20-hydroxyecdysone (**2**) by the fungus *Curvularia lunata* NRRL 2178.[11] 2-Deoxy-ecdysteroids are also a group of minor ecdysteroids isolated from natural sources[12–19] and some of them have been partially synthesized by our group.[20,21] In order to change the mode of the enzymatic reaction that had occurred in the ecdysteroid **2** in our previous biotransformation work,[11] the functional group of the substrate **2** should be modified. Ecdysteroid 2-mesylates have previously been used as the starting material for the synthesis of 2-deoxy-ecdysteroids by catalytic hydrogenolysis[20] and base-catalyzed elimination.[21] In the latter case, the products 3-dehydro-2-deoxy-ecdysteroids were potential intermediates for the synthesis of 2-deoxy-ecdysteroids and related analogues. However, under basic condition they epimerized to the corresponding 5-*epi*-analogues. The milder organic base, guanidine acetate, was found to produce 3-dehydro-2-deoxy-ecdysteroids without epimerization, but the reaction proceeded very sluggishly.[21]



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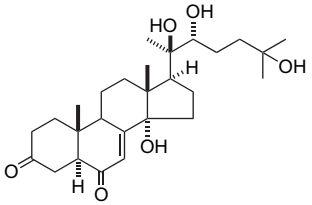
By the same analogy, we would like to investigate whether microbial transformation could be employed in the synthesis of 3-dehydro-2-deoxy-ecdysteroids from the substrates that constituted the same 3-hydroxy-2-mesyloxy functional groups to drive the enzymatic reaction to proceed in similar fashion to that occurred in the previous reported chemical synthesis.[21] This present paper describes the convenient biotransformation of the mesylate **3** to the keto analogue **4** by *C. lunata* NRRL 2178 without epimerization to the corresponding 5-*epi*-analogue **5**. A number of other ecdysteroid analogues have also been subjected to similar biotransformations.



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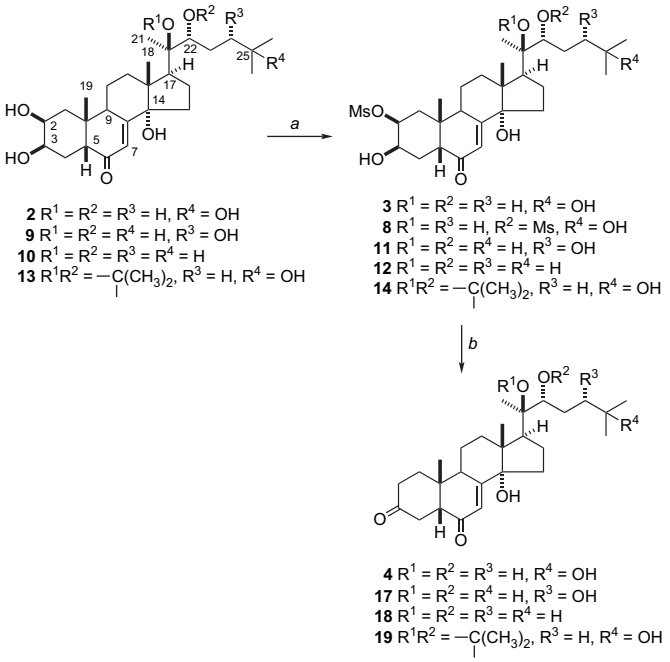
2. Results and discussion

2.1. Synthesis of ecdysteroid 2-mesylates

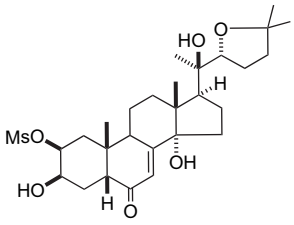
A number of ecdysteroid 2-mesylates have been synthesized as substrates for microbial transformations. The synthesis of 20-hydroxyecdysone 2-mesylate **3** is shown in Scheme 1. The



Scheme 1. Synthesis of the mesylate derivatives **3**, **11**, **12**, and **14** and microbial transformation of the mesylates to the 3-dehydro-2-deoxy-ecdysteroids. Reagents and conditions: *a*, $MeSO_2Cl$/pyridine/$CHCl_3$, 0–5 °C, then ambient temperature; *b*, *Curvularia lunata* NRRL 2178.

readily available ecdysteroid 20-hydroxyecdysone (**2**)[22] was subjected to selective mesylation to the corresponding mesylate **3** (60%) and the minor product **6** (19%). The ^1H and ^{13}C NMR values of **3** were assigned on the basis of COSY, DEPT, HMQC, and HMBC spectra and comparison with the reported values of related compounds. The HR-TOFMS (APCI, negative ion mode) exhibited a pseudo-molecular ion at *m/z* 593.2556 $[M+Cl]^-$ corresponding to the molecular formula $C_{28}H_{46}O_9S$. As expected, the equatorial 2-hydroxyl group was more readily mesylated than the axial 3-hydroxyl group. The downfield shift of the H-2 signal in the starting compound **2** (data not shown) to δ 4.84 in the mesylate **3**, and the appearance of OMs signal of **3** at δ 3.05 indicated that mesylation has taken place at the 2-position. The ^{13}C NMR spectrum indicated the presence of the methyl resonance of the mesyloxyl group at δ 38.8 and the relative downfield signal (δ 80.7) of C-2. The result was in agreement with the reported acetylation rate study.[23] Compound **6** showed similar ^1H NMR data to those of **3**. The ESMS and HR-TOFMS data (see Section 3) of compound **6** indicated a 18 mass unit less than the major product **3**, suggesting that **6** might be a cyclic product obtained through the intermediacy of the epoxide **7**,[24] which in turn was obtained from 20-hydroxyecdysone 2,22-dimesylate (**8**). The second possibility was the formation of an intermediate with C-25 carbocation, followed by the attack of the 22-hydroxyl group to yield **6**. However, the first mechanism seemed to be more likely, since the intermediacy of a 22-mesylate had been observed in the formation of the tetrahydrofuran ring at the side chain of ecdysteroids.[24] From the spectroscopic data, compound **6** was concluded to be shidasterone 2-mesylate. It should be noted that the third mechanism involving the attack of the 25-hydroxyl group at the 22-position

of **8** in the S_N2 fashion was unlikely, since inversion of configuration at C-22 should have occurred. In order to see whether the side chain would exert any effect on biotransformation of the mesylate, compound **6** was also used as a substrate in this study.



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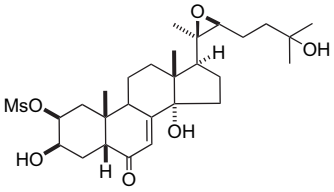

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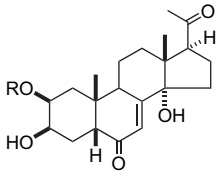
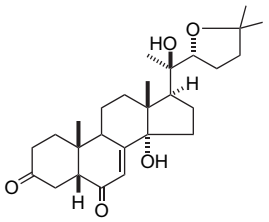
Starting from pterosterone (**9**) and ponasterone A (**10**), which were, respectively, isolated from *Vitex glabrata*[25] and partially synthesized from **2** by the literature method,[26] the corresponding mesylates **11** and **12** were prepared in the same manner as that of **3** (Scheme 1). Furthermore, 20-hydroxyecdysone 20,22-acetonide (**13**) was also used to prepare the corresponding mesylate **14** in 90% yield by the literature method.[20] In order to further study the effect of the side chain on biotransformation process, poststerone (**15**), which was obtained by the literature oxidation of compound **2** with CrO_3–pyridine,[26] was similarly mesylated to compound **16** in 85% yield. The structure of **16** was established from the spectroscopic data (see Section 3).



15 R = H
16 R = Ms

2.2. Biotransformation of ecdysteroid 2-mesylates

Biotransformation of the mesylates **3**, **11**, **12**, and **14** by *C. lunata* NRRL 2178 gave 3-dehydro-2-deoxy-20-hydroxyecdysone (**4**), 3-dehydro-2-deoxy-pterosterone (**17**), 3-dehydro-2-deoxy-ponasterone A (**18**), and 3-dehydro-2-deoxy-20-hydroxyecdysone 20,22-acetonide (**19**) in 60, 30, 34, and 48% yields, respectively, based on the unrecovered substrates (Scheme 1). The mesylates **6** and **16** were similarly biotransformed to 3-dehydro-2-deoxy-shidasterone (**20**) and 3-dehydro-2-deoxyposterone (**21**) in 50 and 92% yields, respectively. The structures of the biotransformation products were determined from IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data.



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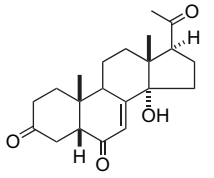

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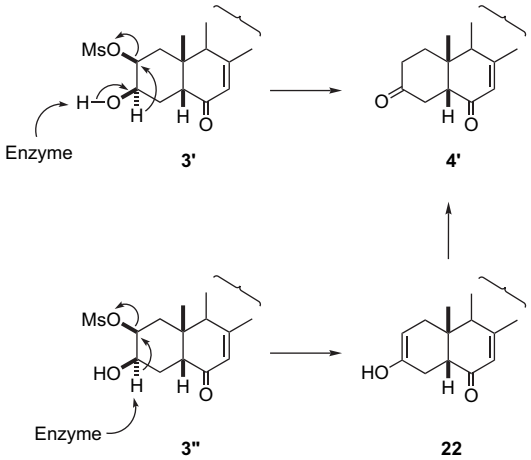
Compound **4** was obtained as the sole biotransformation product from the mesylate **3** by *C. lunata* NRRL 2178 in reasonably good yield (60% based on the unrecovered starting material **2**). The pseudo-molecular ion $[M-H]^-$ at *m*/*z* 461 in the ESMS was compatible with a molecular formula $C_{27}H_{42}O_6$. The ^{1}H and ^{13}C NMR values of **4** were assigned on the basis of COSY, DEPT, HMQC, and HMBC spectra and comparison with those of the reported values. Apart from the presence of the unsaturated keto absorption band at 1659 cm^{-1} in the IR spectrum, a new saturated keto group was evident from the IR absorption band at 1717 cm^{-1}. In addition to the unsaturated keto resonance at δ 199.0 in the ^{13}C NMR spectrum, a new saturated keto resonance appeared at δ 207.7. The absence of the mesyloxyl and the carbinol protons at the C-2 and C-3 positions around δ 3.0, 4.8, and 4.2, respectively, in the ^{1}H NMR spectrum was consistent with the structure **4**. Placement of the saturated keto function at the C-3 position of the A-ring, instead of the C-2 position, was based on mechanistic consideration as shown in Scheme 2. The proposed mechanism to form the 3-dehydro-2-deoxy ecdysteroid would involve abstraction of a proton from the 3-hydroxyl group of the substrate **3** (presented as the partial structure **3′**) followed by a hydride shift to displace the leaving



Scheme 2. Possible mechanisms for the microbial transformation of 20-hydroxyecdysone 2-mesylate (**3**) to 3-dehydro-2-deoxy-20-hydroxyecdysone (**4**) by *Curvularia lunata* NRRL 2178.

mesyloxyl group to yield the corresponding keto analogue **4** (presented as the partial structure **4′**). Alternative possible mechanism would involve abstraction of the proton at the 3-position of **3** (presented as the partial structure **3″**) followed by elimination of the mesyloxyl group to yield the enol **22**, which would then tautomerize to **4′**. In order to assume the trans-diaxial orientation of the 2-mesyloxyl group and the 3-hydrogen without C-5 epimerization, the chair conformation of the A-ring has to adopt the less preferred boat or twist-boat conformation as that occurred in the reported DBU-catalyzed elimination.[27]

The ^{1}H NMR data of **4** were consistent with those of the compound synthesized by chemical method.[21] It should be mentioned that the reported chemical synthesis of **4** involved guanidine acetate-catalyzed elimination of the mesylate **14** at 50 °C for 16 days, followed by deacetonation. The reported overall yield of **4** from the mesylate **14** was 45%.[21]

Compound **17** was obtained from biotransformation of pterosterone 2-mesylate (**9**). The HR-TOFMS established its molecular formula $C_{27}H_{42}O_6$. The IR absorption band at 1708 cm^{-1} and the ^{13}C NMR resonance at δ 208.1 indicated the presence of a saturated keto group. The ^{1}H NMR spectral features revealed a 3-dehydro-2-deoxy system as that of compound **4**. The only differences were those arising from pterosterone side chain, i.e., the presence of H-22 and H-24 signals as a broad doublet (J=10.2 Hz) at δ 3.67 and multiplet at δ 3.27, together with the doublet (J=6.8 Hz) signals of 26-Me and 27-Me at δ 0.91 and 0.92, respectively. The ^{13}C NMR spectral features of **17** were also similar to those of compound **4**. The differences were those arising from pterosterone side chain (see Section 3). Compound **17** was thus concluded to be 3-dehydro-2-deoxy-pterosterone. As expected, biotransformation of ponasterone A 2-mesylate (**12**), the 25-deoxy analogue of compound **3**, gave 3-dehydro-2-deoxy-ponasterone A (**18**). This compound showed a new saturated keto group as evident from the IR absorption band at 1712 cm^{-1} and the ^{13}C NMR resonance at δ 208.1. The pseudo-molecular ion $[M+Cl]^-$ in the HR-TOFMS was compatible with a molecular formula $C_{30}H_{46}O_6$. The ^{1}H NMR spectral pattern of **18** was very similar to that of compound **4**, except for the signals of the 26-Me and 27-Me, which appeared as two doublets, J=6.2 Hz, at δ 0.88 and 0.89 instead of a singlet at δ 1.35. The ^{13}C NMR spectral features of **18** were also similar to those of compound **4**. The differences were those of the side chain C-24 to C-27 (see Section 3). Compound **18** was thus concluded to be 3-dehydro-2-deoxy-ponasterone A.

To see whether the side chain of the ecdysteroid 2-mesylates would have any effect on biotransformation, the mesylates with the acetonide protecting group (compound **14**), the cyclic ether side chain (compound **6**), and that without the side chain (compound **16**) were subjected to biotransformations by *C. lunata* NRRL 2178. Compound **19** was obtained in 48% yield from the biotransformation of 20-hydroxyecdysone 20,22-acetonide 2-mesylate (**14**). The sodiated molecular ion $[M+Na]^+$ at *m/z* 525 in the ESMS and the negative ion HR-TOFMS at *m/z* 537.2990 $[M+Cl]^-$ established a molecular formula $C_{30}H_{46}O_6$. The ^{1}H NMR data of **19** were consistent with the reported values[21] and the ^{13}C NMR data (see Section 3) were in agreement with the structure. Compound **19** was thus concluded as 3-dehydro-2-deoxy-20-hydroxyecdysone 20,22-acetonide. Compound **20** was obtained from biotransformation of shidasterone 2-mesylate (**6**). The IR spectrum showed absorption band of a saturated keto group at 1721 cm^{-1} and the ^{13}C NMR spectrum exhibited a saturated keto resonance at δ 209.5. Positive ion ESMS at *m/z* 467 $[M+Na]^+$ and positive ion HR-TOFMS at *m/z* 467.2770 $[M+Na]^+$ established a molecular formula $C_{27}H_{40}O_5$. The ^{1}H and ^{13}C NMR features of the nucleus of **20** were similar to those of compounds **4**, **17**, and **18**. The differences were those of the side chain, which were very similar to those of compound **6**. Compound **20** was thus concluded to be 3-dehydro-2-deoxy-shidasterone. Biotransformation of poststerone 2-mesylate (**16**) gave compound **21**. The ESMS and HR-TOFMS of **21** established its molecular formula $C_{21}H_{28}O_4$. The IR spectrum showed absorption bands of two saturated keto groups at 1712 and 1698 cm^{-1} and an unsaturated keto group at 1649 cm^{-1}, corresponding to the ^{13}C resonances at δ 208.0 (C-3), 209.3 (C-20), and 199.2 (C-6). As expected, the ^{1}H NMR features of the nucleus of **21** were similar to those of compounds **4** and **17**–**20**. The different spectral features are those of the side chain, which are very similar to those of compound **16**. The structure of metabolite **21** was thus concluded to be 3-dehydro-2-deoxy-poststerone.

In conclusion, the microbial transformations of ecdysteroids with 3-hydroxy-2-mesyloxy groups to the corresponding 3-dehydro-2-deoxy analogues have been achieved by the fungus *C. lunata* NRRL 2178. The 3-dehydro-2-deoxy-ecdysteroids, which were the only products were obtained in 30–60% yields, except for compound **16**, which was obtained in 92% yield. The bioconversion of **15** to **16** in an excellent yield is worth to note. Since compound **15** was the only substrate without the long side chain, it is possible that the lack of the bulky chain contributed to facile enzymatic conversion of **15** to **16**. However, the existing data did not permit definite conclusion of this microbial transformation. It is also worth to note that the mild bioconversion conditions prevented the metabolites from C-5 epimerization.

3. Experimental

3.1. General experimental procedures

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. IR spectra were recorded in KBr on a Perkin–Elmer FT-IR Spectrum BX spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AVANCE 400 spectrometer. Mass spectra were obtained using a Finnigan LC-Q mass spectrometer. High resolution mass spectra were obtained using a Bruker micrOTOF mass spectrometer. Column chromatography and TLC were carried out using Merck silica gel 60 (<0.063 mm) and precoated silica gel 60 F_{254} plates, respectively. Spots on TLC were detected under UV light (254 nm) and by spraying with anisaldehyde–H_2SO_4 reagent followed by heating.

3.2. Organism

C. lunata NRRL (strain no. 2178) was obtained from NRRL (Illinois, USA). Stock culture of the fungus was maintained on potato dextrose agar slant. It was stored at 4 °C and subcultured monthly at 30 °C.

3.3. Incubation experiments

The stock culture of *C. lunata* NRRL 2178 was maintained on a potato dextrose agar slant. Erlenmeyer flask (250 mL), each containing 100 mL of liquid medium consisting of 0.1% peptone, 0.1% yeast extract, 0.1% beef extract, and 0.5% glucose were inoculated with freshly obtained *C. lunata* cultured from the agar slant on a rotary shaker at 200 rpm. After cultivation at ambient temperature for 72 h, the substrate solution (10 mg of substrate dissolved in 100 μL DMSO and 5 μL Tween 80) was added to each flask, and the incubation continued for 8 days. Culture control consisted of fermentation blank in which *C. lunata* was grown under identical condition but without substrate.

3.4. Synthesis of substrates

3.4.1. Mesylation of compound 2

20-Hydroxyecdysone (**2**) (140 mg) was dissolved in a mixture of pyridine (3 mL) and $CHCl_3$ (1 mL), then mesyl chloride (0.2 mL) was added. The reaction mixture was left to stir at 0–5 °C for 30 min and at ambient temperature for 5 h. The reaction mixture was worked up with 1% $NaHCO_3$ (50 mL) and extracted with EtOAc. The EtOAc layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The crude products were separated and purified by column chromatography using CH_2Cl_2–MeOH (95:5) to give 20-hydroxyecdysone 2-mesylate (**3**) (98 mg, 60%) and shidasterone 2-mesylate (**6**) (31 mg, 19%).

*3.4.1.1. 20-Hydroxyecdysone 2-mesylate (**3**).* Amorphous solid; IR ν_{max} 2966, 2922, 1717, 1655, 1459, 1350, 1200, 1174, 1059, 947, 850 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$+4 drops CD_3OD) δ 0.80 (s, 3H, 18-Me), 0.96 (s, 3H, 19-Me), 1.14 (s, 3H, 21-Me), 1.18 (s, 2×3H, 26-Me and 27-Me), 2.28 (m, 1H, H-17), 2.44 (dd, J=12.9, 4.1 Hz, 1H, H-5), 3.05 (s, 3H, OMs), 3.33 (m, $W_{1/2}$=24 Hz, 1H, H-9), 3.34 (d, J=10.7 Hz, 1H, H-22), 4.20 (br s, 1H, H-3), 4.84 (m, $W_{1/2}$=23 Hz, 1H, H-2), 5.80 (d, J=1.8 Hz, 1H, H-7); ^1H NMR (400 MHz, C_5D_5N) δ 1.06 (s, 3H, 19-Me), 1.17 (s, 3H, 18-Me), 1.40 (s, 2×3H, 26-Me and 27-Me), 1.57 (s, 3H, 21-Me), 2.93 (m, $W_{1/2}$=18 Hz, 1H, H-17), 3.02 (br d, J=12.5 Hz, 1H, H-5), 3.30 (s, 3H, OMs), 3.60 (m, $W_{1/2}$=24 Hz, 1H, H-9), 3.86 (br d, J=7.9 Hz, 1H, H-22), 4.84 (br s, 1H, H-3), 5.29 (obscured signal, H-2), 6.23 (br s, 1H, H-7); ^{13}C NMR (100 MHz, C_5D_5N) δ 17.8 (C-18), 21.1 (C-11),[a] 21.4 (C-16),[a] 21.7 (C-21), 24.1 (C-19), 27.5 (C-23), 29.9 (C-26), 30.3 (C-27), 31.6 (C-15),[b] 31.9 (C-12),[b] 32.6 (C-4), 34.2 (C-9), 35.0 (C-1), 38.8 (OMs), 39.4 (C-10), 42.6 (C-24), 47.3 (C-13), 50.0 (C-17), 50.7 (C-5), 65.8 (C-3), 69.7 (C-25), 76.9 (C-20), 77.6 (C-22), 80.7 (C-2), 84.2 (C-14), 121.8 (C-7), 166.2 (C-8), 202.5 (C-6), ('a' and 'b' stand for assignments that may be reversed for signals with the same superscript); ESMS (−ve) *m/z* (% rel intensity): 557 $[M-H]^-$ (100); HR-TOFMS (APCI, −ve) *m/z* 593.2556 $[M+Cl]^-$ (calcd for $C_{28}H_{46}O_9S$+Cl, 593.2557).

*3.4.1.2. Shidasterone 2-mesylate (**6**).* Amorphous solid; IR ν_{max} 3480, 2966, 1714, 1647, 1454, 1338, 1174, 1058, 946, 847 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.80 (s, 3H, 18-Me), 1.00 (s, 3H, 19-Me), 1.16 (s, 3H, 21-Me), 1.21 (s, 3H, 26-Me), 1.22 (s, 3H, 27-Me), 2.26 (m, 1H, H-17), 2.49 (dd, J=13.3, 4.9 Hz, 1H, H-5), 2.98 (m, $W_{1/2}$=27 Hz, 1H, H-9), 3.07 (s, 3H, OMs), 3.86 (br t, J=7.2 Hz, 1H, H-22), 4.28 (br s, 1H, H-3), 4.89 (m, $W_{1/2}$=20 Hz, 1H, H-2), 5.40 (d, J=2.2 Hz, 1H, H-7); ^1H NMR (400 MHz, C_5D_5N) δ 1.06 (s, 3H, 18-Me), 1.07 (s, 3H, 19-Me), 1.18 (s, 3H, 26-Me), 1.19 (s, 3H, 27-Me), 1.41 (s, 3H, 21-Me), 2.75 (br t, J=8.3 Hz, 1H, H-17), 3.24 (s, 3H, OMs), 3.64 (m, $W_{1/2}$=20 Hz, 1H, H-9), 4.09 (br t, J=7.4 Hz, H-22), 4.50 (br s, 1H, H-3), 5.25 (obscured signal, H-2), 6.23 (br s, 1H, H-7); ^{13}C NMR (100 MHz, C_5D_5N) δ 17.8 (C-18), 21.1 (C-11),[a] 21.2 (C-21), 21.6 (C-16),[a] 24.0 (C-19), 27.8 (C-23), 28.3 (C-26), 28.8 (C-27), 31.6 (C-12 and C-15), 32.6 (C-4), 34.1 (C-9), 35.1 (C-1), 38.7 (OMs), 39.0 (C-24), 39.4 (C-10), 47.6 (C-13), 50.7 (C-17), 51.4 (C-5), 65.8 (C-3), 75.7 (C-25), 80.5 (C-20), 80.7 (C-2), 84.1 (C-14), 85.0 (C-22), 121.9 (C-7), 166.2 (C-8), 202.4 (C-6), ('a' stands for assignments that may be reversed for signals with the same superscript); ESMS (−ve) *m/z* (% rel intensity): 539 $[M-H]^-$ (100); HR-TOFMS (ESI, +ve) *m/z* 563.2648 $[M+Na]^+$ (calcd for $C_{28}H_{44}O_8S$+Na, 563.2649).

3.4.2. Mesylation of compounds 9, 10, 13, and 15

Compound **9** (37 mg), **10** (63 mg), and **15** (50 mg) were separately subjected to mesylation in similar manner to that of compound **2** to give the mesylates **11** (30 mg, 70%), **12** (55 mg, 75%), and **16** (51.5 mg, 85%). The mesylate **14** (47 mg) was similarly prepared in 90% yield from **13** (45 mg),[20] which in turn was prepared from **2** by the literature method.[28]

*3.4.2.1. Pterosterone 2-mesylate (**11**).* Amorphous solid; IR ν_{max} 3447, 2962, 1654, 1340, 1174, 942 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H, 18-Me), 0.90 (d, J=6.2 Hz, 3H, 26-Me), 0.91 (s, 3H, 27-Me), 0.99 (s, 3H, 19-Me), 1.18 (s, 3H, 21-Me), 2.26 (br t, J=8.2 Hz, 3H, H-17), 2.47 (br d, J=12.5 Hz, 1H, H-5), 3.01 (m, $W_{1/2}$=22 Hz, 1H, H-9), 3.05 (s, 3H, OMs), 3.58 (m, $W_{1/2}$=18 Hz, 1H, H-24), 3.64 (br d, J=10.4 Hz, 1H, H-22), 4.24 (br s, 1H, H-3), 4.85 (br d, J=11.4 Hz, 1H, H-2), 5.87 (br s, 1H, H-7); ESMS (+ve) *m/z* (% rel intensity): 581 $[M+Na]^+$ (100); HR-TOFMS (APCI, −ve) *m/z* 593.2553 $[M+Cl]^-$ (calcd for $C_{28}H_{46}O_9S$+Cl, 593.2557).

*3.4.2.2. Ponasterone A 2-mesylate (**12**).* Amorphous solid; IR ν_{max} 3443, 2956, 1655, 1448, 1341, 1174, 1075, 917 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.77 (s, 3H, 18-Me), 0.81 (d, J=6.0 Hz, 3H, 26-Me), 0.82 (d, J=6.0 Hz, 3H, 27-Me), 0.93 (s, 3H, 19-Me), 1.10 (s, 3H, 21-Me), 2.26 (dd, J=9.0, 8.3 Hz, 1H, H-17), 2.42 (dd, J=13.4, 3.9 Hz, 1H, H-5), 2.93 (m, $W_{1/2}$=27 Hz, 1H, H-9), 2.99 (s, 3H, OMs), 3.31 (br d, J=

9.6 Hz, 1H, H-22), 4.20 (br s, 1H, H-3), 4.80 (ddd, J=10.8, 4.2, 3.0 Hz, 1H, H-2), 5.77 (br d, J=2.1 Hz, 1H, H-7); ESMS (+ve) *m/z* (% rel intensity): 565 $[M+Na]^+$ (100); HR-TOFMS (APCI, −ve) *m/z* 577.2607 $[M+Cl]^-$ (calcd for $C_{28}H_{46}O_8S+Cl$, 577.2607).

*3.4.2.3. Poststerone 2-mesylate (**16**).* Powder, mp 170–172 °C; IR ν_{max} 3406, 2952, 2885, 1694, 1642, 1439, 1358, 1175, 1078, 1059, 951, 902, 846 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.58 (s, 3H, 18-Me), 0.98 (s, 3H, 19-Me), 2.12 (s, 3H, 21-Me), 2.48 (dd, J=13.1, 3.7 Hz, H-5), 3.04 (obscured signal, 1H, H-9), 3.07 (s, 3H, OMs), 4.25 (br s, 1H, H-3), 4.87 (m, $W_{1/2}$= 22 Hz, 1H, H-2), 5.83 (br d, J=1.9 Hz, 1H, H-7); ^1H NMR (400 MHz, C_5D_5N) δ 0.66 (s, 3H, 18-Me), 1.02 (s, 3H, 19-Me), 1.62 (m, $W_{1/2}$=24 Hz, 1H, H-11a), 1.75 (br d, J=12.2 Hz, 1H, H-12a), 1.92 (obscured signal, 1H, H-16a), 2.15 (s, 3H, 21-Me), 2.20 (d, J=13.0 Hz, 1H, H-1a), 2.30 (dd, J=13.0, 3.3 Hz, 1H, H-1b), 2.45 (obscured signal, 1H, H-16b), 2.55 (td, J=12.7, 4.1 Hz, 1H, H-12b), 3.04 (br d, J=10.5 Hz, 1H, H-5), 3.25 (s, 3H, OMs), 3.52 (br t, J=8.0 Hz, 1H, H-17), 3.61 (m, $W_{1/2}$=24 Hz, 1H, H-9), 4.51 (br s, 1H, H-3), 5.25 (obscured signal, 1H, H-2), 6.17 (br s, 1H, H-7); ^{13}C NMR (100 MHz, C_5D_5N) δ 17.0 (C-18), 21.0 (C-11), 21.7 (C-16), 23.9 (C-19), 30.3 (C-12), 31.4 (C-21), 31.7 (C-15), 32.5 (C-4), 34.0 (C-9), 35.0 (C-1), 38.7 (OMs), 39.4 (C-10), 47.9 (C-13), 50.6 (C-5), 59.4 (C-17), 65.7 (C-3), 80.5 (C-2), 83.8 (C-14), 122.3 (C-7), 164.6 (C-8), 202.2 (C-6), 209.2 (C-20); ESMS (+ve) *m/z* (% rel intensity): 463 $[M+Na]^+$ (100); HR-TOFMS (APCI, −ve) *m/z* 475.1550 $[M+Cl]^-$ (calcd for $C_{22}H_{32}O_7S+Cl$, 475.1563).

3.5. Biotransformation of 20-hydroxyecdysone 2-mesylate (3)

20-Hydroxyecdysone 2-mesylate (**3**) (5×10 mg) was fed to *C. lunata* NRRL 2178 in Erlenmeyer flasks (5×250 mL), each containing 100 ml of the same medium as outlined above. After 8 days the culture was filtered, the mycelium was washed with EtOAc, and the broth was extracted with EtOAc (3×75 mL). The organic phase was washed with water, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude extract (78 mg) was subjected to column chromatography eluting with CH_2Cl_2–MeOH (95:5) to yield **4** (15 mg, 60% based on the unrecovered starting material) and the starting material **2** (20 mg).

*3.5.1. 3-Dehydro-2-deoxy-20-hydroxyecdysone (**4**)*

Powders from MeOH–$CHCl_3$, mp 222–224 °C (lit.[21] 225–226 °C); IR ν_{max} 3422, 2963, 1717, 1659, 1455, 1384, 1299, 1215, 1153, 1072, 952, 891 cm^{-1}; ^1H NMR (400 MHz, C_5D_5N) δ 1.02 (s, 3H, 19-Me), 1.21 (s, 3H, 18-Me), 1.35 (s, 2×3H, 26-Me and 27-Me), 1.58 (s, 3H, 21-Me), 2.30 (overlapping signal, 1H, H-4a), 2.63 (obscured signal, 1H, H-4b), 2.57 (obscured signal, 1H, H-5), 3.02 (t, J=8.9 Hz, 1H, H-17), 3.60 (m, $W_{1/2}$= 22 Hz, 1H, H-9), 3.87 (br d, J=9.3 Hz, 1H, H-22), 6.18 (br s, 1H, H-7); ^{13}C NMR (100 MHz, C_5D_5N) δ 17.6 (C-18), 21.3 (C-16), 21.4 (C-21), 21.9 (C-11), 22.3 (C-19), 27.2 (C-23), 29.7 (C-26), 29.9 (C-27), 31.1 (C-15), 32.0 (C-12), 34.6 (C-1), 36.0 (C-9), 36.5 (C-10), 36.9 (C-2), 39.0 (C-4), 42.4 (C-24), 48.9 (C-13), 49.9 (C-17), 55.1 (C-5), 69.3 (C-25), 76.5 (C-20), 77.3 (C-22), 84.2 (C-14), 120.1 (C-7), 166.0 (C-8), 199.0 (C-6), 207.7 (C-3); ESMS (−ve) *m/z* (% rel intensity): 461 $[M-H]^-$ (100).

3.6. Biotransformation of pterosterone 2-mesylate (11)

Pterosterone 2-mesylate (**11**) (3×10 mg) was subjected to biotransformation in the similar manner as for compound **3**. The crude extract was chromatographed to yield **17** (5.5 mg, 30% based on the unrecovered starting material) and the starting material **11** (8 mg).

*3.6.1. 3-Dehydro-2-deoxy-pterosterone (**17**)*

Feather-like crystals from EtOAc–hexane, mp 134–136 °C; IR ν_{max} 3422, 2960, 2870, 1708, 1655, 1461, 1384, 1310, 1015, 952, 846 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 3H, 18-Me), 0.91 (d, J=6.8 Hz, 3H, 26-Me), 0.92 (d, J=6.8 Hz, 3H, 27-Me), 1.06 (s, 3H, 19-Me), 1.26 (s, 3H, 21-Me), 3.27 (m, $W_{1/2}$=23 Hz, 1H, H-9), 3.61 (br dd, J=9.1, 4.3 Hz, 1H, H-24), 3.67 (br d, J=10.2 Hz, 1H, H-22), 5.82 (br d, J=1.7 Hz, 1H, H-7); ^1H NMR (400 MHz, C_5D_5N) δ 1.02 (d, J=6.7 Hz, 3H, 26-Me), 1.04 (d, J=6.7 Hz, 3H, 27-Me), 1.05 (s, 3H, 19-Me), 1.22 (s, 3H, 18-Me), 1.61 (s, 3H, 21-Me), 2.96 (br t, J= 7.9 Hz, 1H, H-17), 3.62 (m, $W_{1/2}$=21 Hz, 1H, H-9), 3.96 (m, $W_{1/2}$=19 Hz, 1H, H-24), 4.14 (br d, J=10.1 Hz, 1H, H-22), 6.20 (br s, 1H, H-7); ^{13}C NMR (100 MHz, C_5D_5N) δ 17.0 (C-26), 17.9 (C-18), 19.6 (C-27), 21.6 (C-16), 21.7 (C-21), 22.2 (C-11), 22.6 (C-19), 31.4 (C-15), 32.4 (C-12), 34.0 (C-25), 34.9 (C-1), 35.9 (C-23), 36.2 (C-9), 36.8 (C-10), 37.2 (C-2), 39.3 (C-4), 49.2 (C-13), 50.1 (C-17), 55.5 (C-5), 76.81 (C-20), 76.86 (C-24), 77.6 (C-22), 84.5 (C-14), 120.6 (C-7), 165.7 (C-8), 199.4 (C-6), 208.1 (C-3); ESMS (+ve) *m/z* (% rel intensity): 485 $[M+Na]^+$ (100); HR-TOFMS (APCI, −ve) *m/z* 497.2670 $[M+Cl]^-$ (calcd for $C_{27}H_{42}O_6+Cl$, 497.2675).

3.7. Biotransformation of ponasterone A 2-mesylate (12)

Ponasterone A 2-mesylate (**12**) (5×10 mg) was subjected to biotransformation in the same manner as for compound **3**. The crude extract was chromatographed to yield **18** (8.5 mg, 34% based on the unrecovered starting material) and the starting material **12** (20 mg).

*3.7.1. 3-Dehydro-2-deoxy-ponasterone A (**18**)*

Prisms from CH_2Cl_2–hexane, mp 177–179 °C; IR ν_{max} 3420, 2954, 2870, 1712, 1660, 1465, 1384, 1306, 1251, 908 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (d, J=6.2 Hz, 3H, 26-Me), 0.88 (s, 3H, 18-Me), 0.89 (d, J=6.2 Hz, 3H, 27-Me), 1.07 (s, 3H, 19-Me), 1.18 (s, 3H, 21-Me), 3.28 (m, $W_{1/2}$= 23 Hz, 1H, H-9), 3.39 (br d, J=8.5 Hz, 1H, H-22), 5.86 (br d, J=2.2 Hz, H-7); ^1H NMR (400 MHz, C_5D_5N) δ 0.81 (d, J=6.1 Hz, 3H, 26-Me), 0.82 (d, J=6.1 Hz, 3H, 27-Me), 1.05 (s, 3H, 19-Me), 1.23 (s, 3H, 18-Me), 1.59 (s, 3H, 21-Me), 2.60 (dd, J=11.1, 4.1 Hz, 1H, H-5), 2.96 (t, J=9.1 Hz, 1H, H-17), 3.65 (m, $W_{1/2}$=26 Hz, 1H, H-9), 3.81 (d, J=10.2 Hz, 1H, H-22), 6.20 (br s, 1H, H-7); ^{13}C NMR (100 MHz, C_5D_5N) δ 17.9 (C-18), 21.61 (C-16), 21.65 (C-21), 22.2 (C-11), 22.4

(C-26), 22.6 (C-27), 23.3 (C-19), 28.2 (C-25), 30.3 (C-23), 31.4 (C-15), 32.4 (C-12), 34.9 (C-1), 36.2 (C-9), 36.8 (C-10), 37.2 (C-2 and C-24), 39.3 (C-4), 49.2 (C-13), 50.1 (C-17), 55.5 (C-5), 76.8 (C-20), 76.9 (C-22), 84.5 (C-14), 120.5 (C-7), 166.9 (C-8), 199.3 (C-6), 208.1 (C-3); ESMS (+ve) *m/z* (% rel intensity): 915 $[2M+Na]^+$ (100); HR-TOFMS (APCI, −ve) *m/z* 481.2722 $[M+Cl]^-$ (calcd for $C_{27}H_{42}O_5$+Cl, 481.2726).

*3.8. Biotransformation of 20-hydroxyecdysone 20,22-acetonide 2-mesylate (**14**)*

20-Hydroxyecdysone 20,22-acetonide 2-mesylate (**14**) (4×10 mg) was subjected to biotransformation in similar manner as for compound **3**. The crude extract was chromatographed to yield **19** (14 mg, 48% based on the unrecovered starting material) and the starting material **14** (5 mg).

*3.8.1. 3-Dehydro-2-deoxy-20-hydroxyecdysone 20,22-acetonide (**19**)*

Foam; IR ν_{max} 3441, 2967, 1712, 1666, 1453, 1372, 1252, 1217, 1170, 1105, 1002, 905 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.80 (s, 3H, 18-Me), 1.06 (s, 3H, 19-Me), 1.15 (s, 3H, 21-Me), 1.22 (s, 2×3H, 26-Me and 27-Me), 1.31, 1.40 (each s, 2×3H, acetonide Me), 2.25 (dd, *J*=9.2, 7.9 Hz, 1H, H-17), 2.31 (dd, *J*=11.7, 3.8 Hz, 1H, H-5), 3.26 (m, $W_{1/2}$=21 Hz, 1H, H-9), 3.64 (br d, *J*=8.4 Hz, 1H, H-22), 5.86 (br d, *J*=2.1 Hz, 1H, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1 (C-18), 21.2 (C-11 and C-16), 21.8 (C-21), 22.6 (C-19), 23.5 (C-23), 26.8 (acetonide Me), 28.9 (acetonide Me), 29.2 (C-27), 29.6 (C-26), 31.2 (C-12),[a] 31.6 (C-15),[a] 34.8 (C-1), 35.1 (C-9), 36.6 (C-10), 36.9 (C-2), 39.1 (C-4), 41.3 (C-24), 48.0 (C-13), 49.0 (C-17), 55.4 (C-5), 70.4 (C-25), 82.0 (C-22), 84.3 (C-14), 85.3 (C-20), 107.0 (acetonide C), 120.7 (C-7), 164.3 (C-8), 199.3 (C-6), 208.2 (C-3), ('a' stands for assignments that may be reversed for signals with the same superscript); ESMS (+ve) *m/z* (% rel intensity) 525 $[M+Na]^+$ (100); HR-TOFMS (APCI, −ve) *m/z* 537.2990 $[M+Cl]^-$ (calcd for $C_{30}H_{46}O_6$+Cl, 537.2988).

*3.9. Biotransformation of shidasterone 2-mesylate (**6**)*

Shidasterone 2-mesylate (**6**) (2×10 mg) was subjected to biotransformation in similar manner as for compound **3**. The crude extract was chromatographed to yield **20** (4.5 mg, 50% based on the unrecovered starting material) and compound **6** (9 mg).

*3.9.1. 3-Dehydro-2-deoxy-shidasterone (**20**)*

Needles from CH_2Cl_2–hexane, mp 227–229 °C; IR ν_{max} 3445, 2967, 2922, 1721, 1652, 1454, 1382, 1126, 952, 871 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (s, 3H, 18-Me), 1.06 (s, 3H, 19-Me), 1.18 (s, 3H, 21-Me), 1.21 (s, 3H, 26-Me), 1.22 (s, 3H, 27-Me), 3.26 (m, $W_{1/2}$=24 Hz, 1H, H-9), 3.86 (br t, *J*=7.1 Hz, H-22), 5.86 (br d, *J*=1.7 Hz, 1H, H-7); ^1H NMR (400 MHz, C_5D_5N) δ 1.05 (s, 3H, 18-Me), 1.08 (s, 3H, 19-Me), 1.20 (s, 3H, 26-Me), 1.21 (s, 3H, 27-Me), 1.42 (s, 3H, 21-Me), 2.61 (dd, *J*=11.7, 3.8 Hz, 1H, H-5), 2.85 (t, *J*=8.7 Hz, 1H, H-17), 3.63 (m, $W_{1/2}$=26 Hz, 1H, H-9), 4.10 (t, *J*=7.4 Hz, 1H, H-22), 6.18 (br s, 1H, H-7); ^{13}C NMR (100 MHz, C_5D_5N) δ 17.9 (C-18), 21.2 (C-21), 21.8 (C-16),[a] 22.1 (C-11),[a] 22.6 (C-19), 27.7 (C-23), 28.3 (C-26), 28.8 (C-27), 31.3 (C-15),[b] 32.1 (C-12),[b] 34.9 (C-1), 36.1 (C-9), 36.8 (C-10), 37.2 (C-2), 38.9 (C-24), 39.3 (C-4), 48.7 (C-13), 51.1 (C-17), 55.4 (C-5), 75.6 (C-25), 80.4 (C-20), 84.4 (C-14), 85.0 (C-22), 120.4 (C-7), 176.6 (C-8), 198.2 (C-6), 209.5 (C-3), ('a' and 'b' stand for assignments that may be reversed for signals with the same superscript); ESMS (+ve) *m/z* (% rel intensity): 467 $[M+Na]^+$ (100); HR-TOFMS (ESI, +ve) *m/z* 467.2770 $[M+Na]^+$ (calcd for $C_{27}H_{40}O_5$+Na, 467.2768).

*3.10. Biotransformation of poststerone 2-mesylate (**16**)*

Poststerone 2-mesylate (**16**) (3×10 mg) was subjected to biotransformation in similar manner as for compound **3**. The crude extract was chromatographed to yield **21** (14 mg, 92% based on the unrecovered starting material) and the starting material **16** (10.5 mg).

*3.10.1. 3-Dehydro-2-deoxy-poststerone (**21**)*

Amorphous solid; IR ν_{max} 3456, 2961, 2878, 1712, 1698, 1649, 1421, 1386, 1310, 1279, 1259, 1169, 1130, 1079, 1015, 912, 888 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.63 (s, 3H, 18-Me), 1.06 (s, 3H, 19-Me), 2.14 (s, 3H, 21-Me), 3.30 (overlapping signal, 2×1H, H-9 and H-17), 5.86 (br d, *J*=2.2 Hz, 1H, H-7); ^1H NMR (400 MHz, C_5D_5N) δ 0.70 (s, 3H, 18-Me), 1.02 (s, 3H, 19-Me), 1.37 (ddd, *J*=14.1, 11.1, 5.1 Hz, 1H, H-1a), 1.61 (obscured signal, 1H, H-11a), 1.85 (partially overlapping signal, 1H, H-11b), 1.96 (obscured signal, 1H, H-1b), 2.16 (s, 3H, 21-Me), 2.35 (overlapping signal, 1H, H-4a), 2.61 (dd, *J*=11.2, 4.5 Hz, 1H, H-5), 2.72 (dd, *J*=14.5, 11.2 Hz, 1H, H-4b), 3.60 (overlapping signal, 2×1H, H-9 and H-17), 6.14 (br d, *J*=1.6 Hz, 1H, H-7); ^{13}C NMR (100 MHz, C_5D_5N) δ 17.2 (C-18), 22.0 (C-16), 22.2 (C-11), 22.6 (C-19), 30.8 (C-15), 31.3 (C-21), 31.5 (C-12), 34.8 (C-1), 36.3 (C-9), 36.8 (C-10), 37.1 (C-2), 39.2 (C-4), 49.2 (C-13), 55.2 (C-5), 59.5 (C-17), 84.2 (C-14), 120.8 (C-7), 165.3 (C-8), 199.2 (C-6), 208.0 (C-3), 209.3 (C-20); ESMS (+ve) *m/z* (% rel intensity): 711 $[2M+Na]^+$ (100); HR-TOFMS (APCI, −ve) *m/z* 379.1687 $[M+Cl]^-$ (calcd for $C_{21}H_{28}O_4$+Cl, 379.1682).

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