

## Supporting materials

**article title:** Steroid Metabolism in Thermophilic Actinobacteria *Saccharopolyspora hirsuta* VKM Ac-666<sup>T</sup>

**journal name:** Microorganisms

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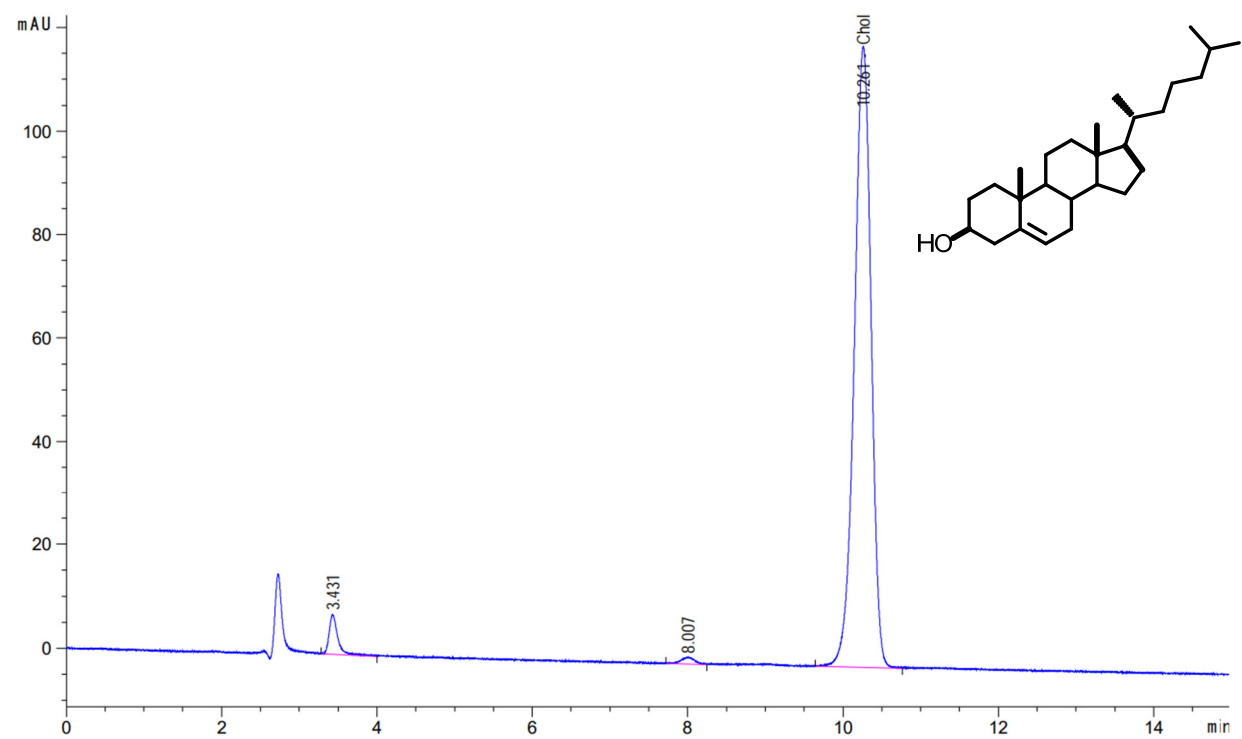
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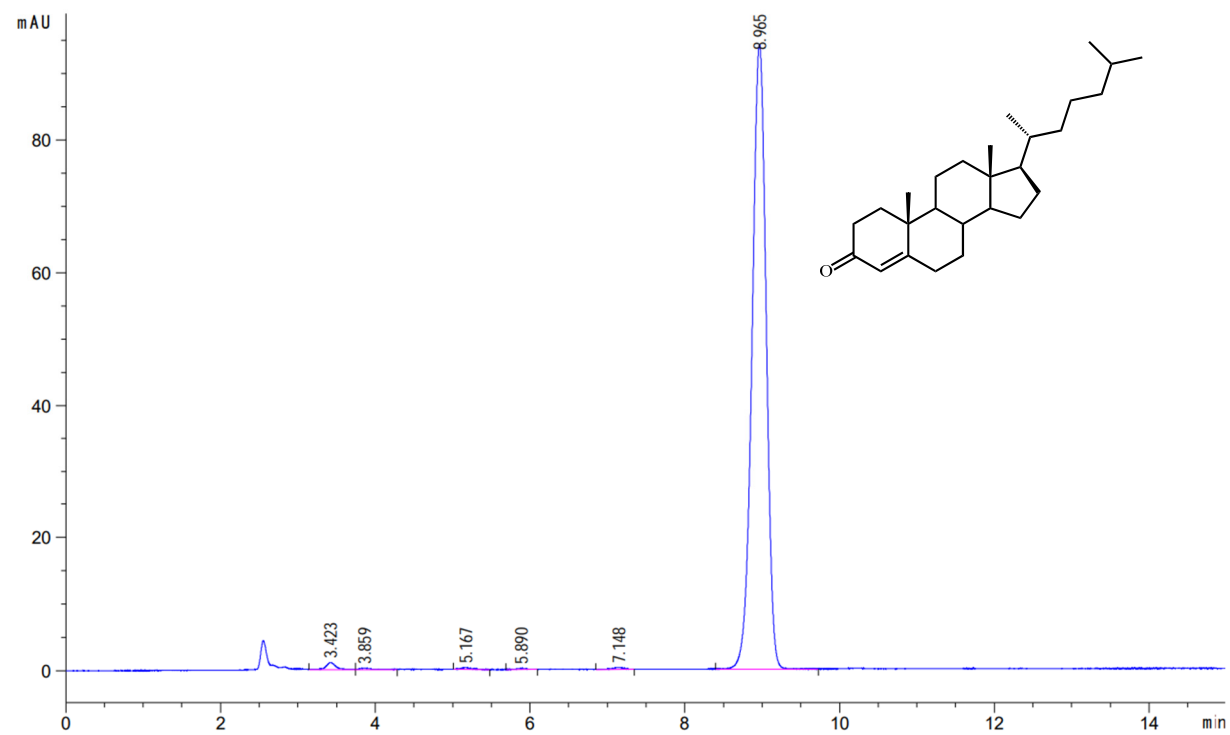
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**Figure S1. HPLC-profile (mobile phase acetonitrile:2-propanol:water (50:45:5 v/v/v/))**



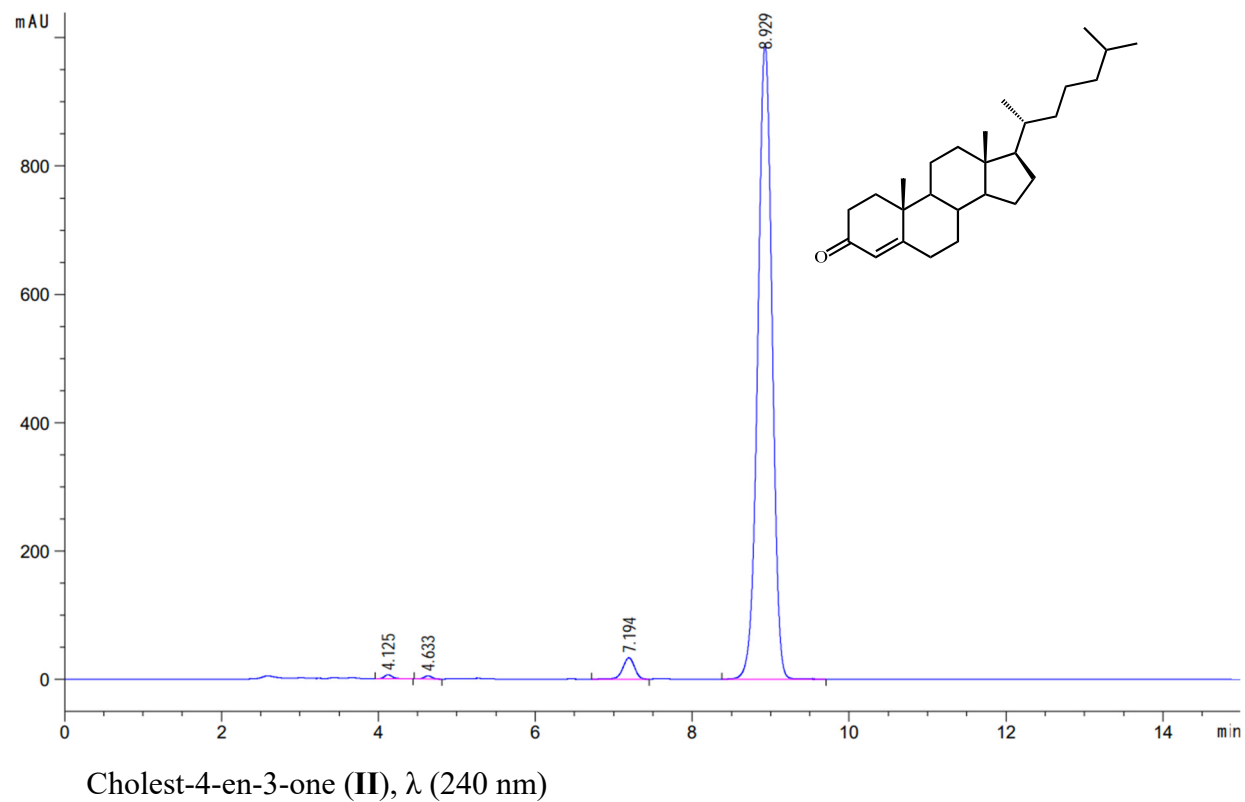
Cholesterol (standard),  $\lambda(200\text{ nm})$

**Figure S2. HPLC-profile (mobile phase acetonitrile:2-propanol:water (50:45:5 v/v/v/))**

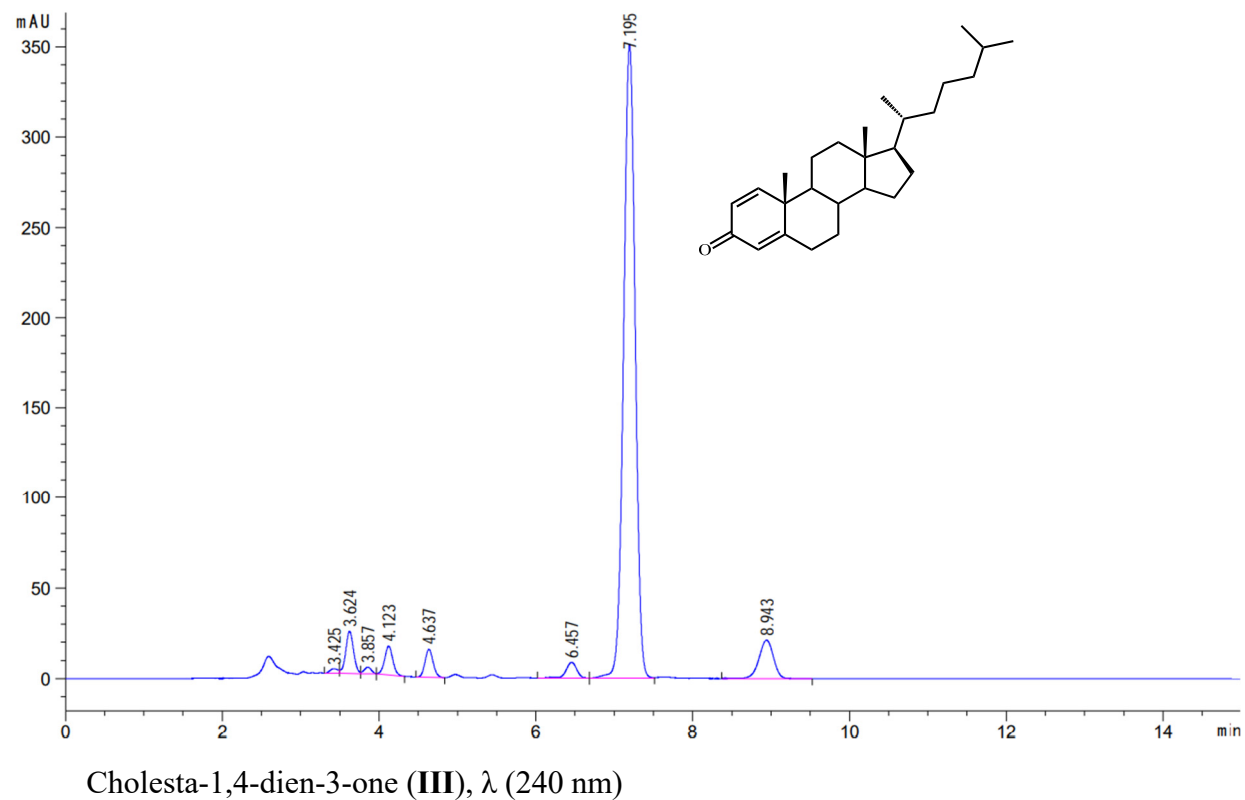


Cholest-4-en-3-one (cholestenone) (standard),  $\lambda$  (240 nm)

**Figure S3. HPLC-profile (mobile phase acetonitrile:2-propanol:water (50:45:5 v/v/v))  
of the individual compound isolated from the transformation media at the cholesterol conversion by *S.hirsuta***

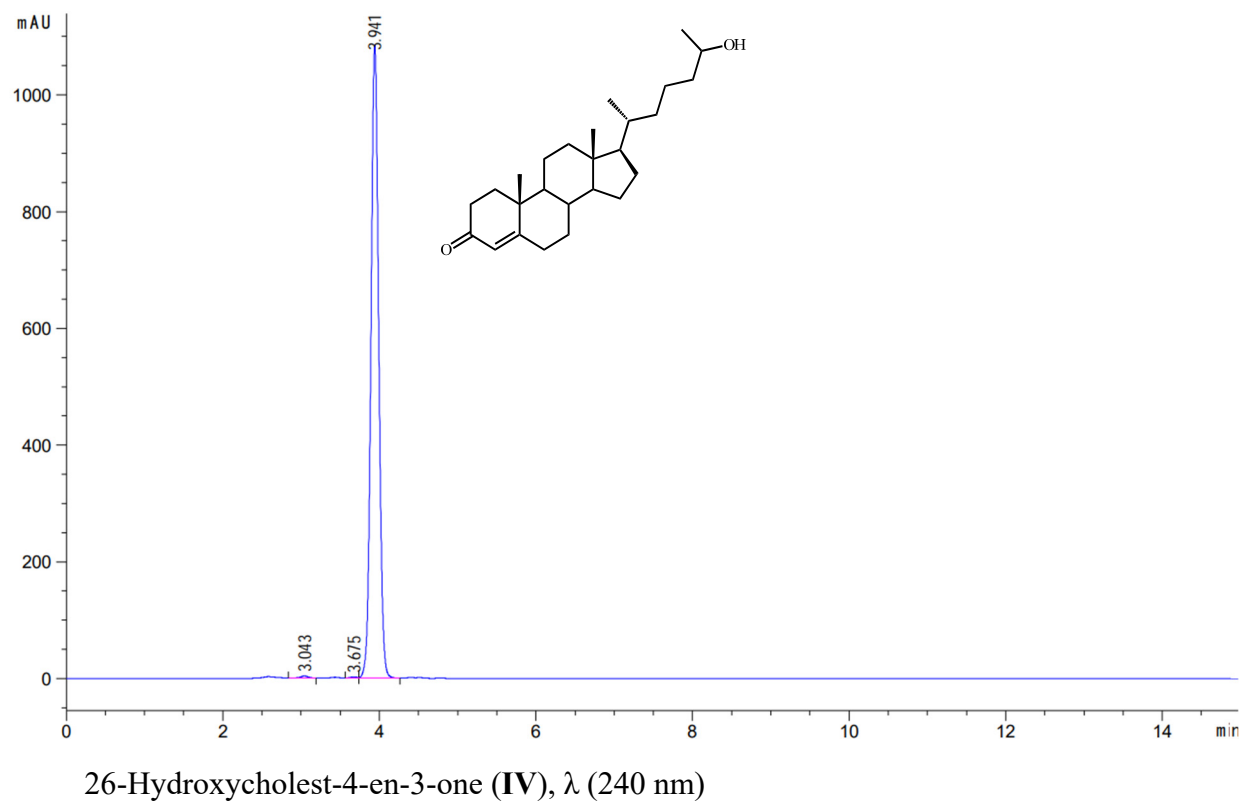


**Figure S4. HPLC-profile (mobile phase acetonitrile:2-propanol:water (50:45:5 v/v/v))  
of the individual compound isolated from the transformation media at the cholesterol conversion by *S.hirsuta***

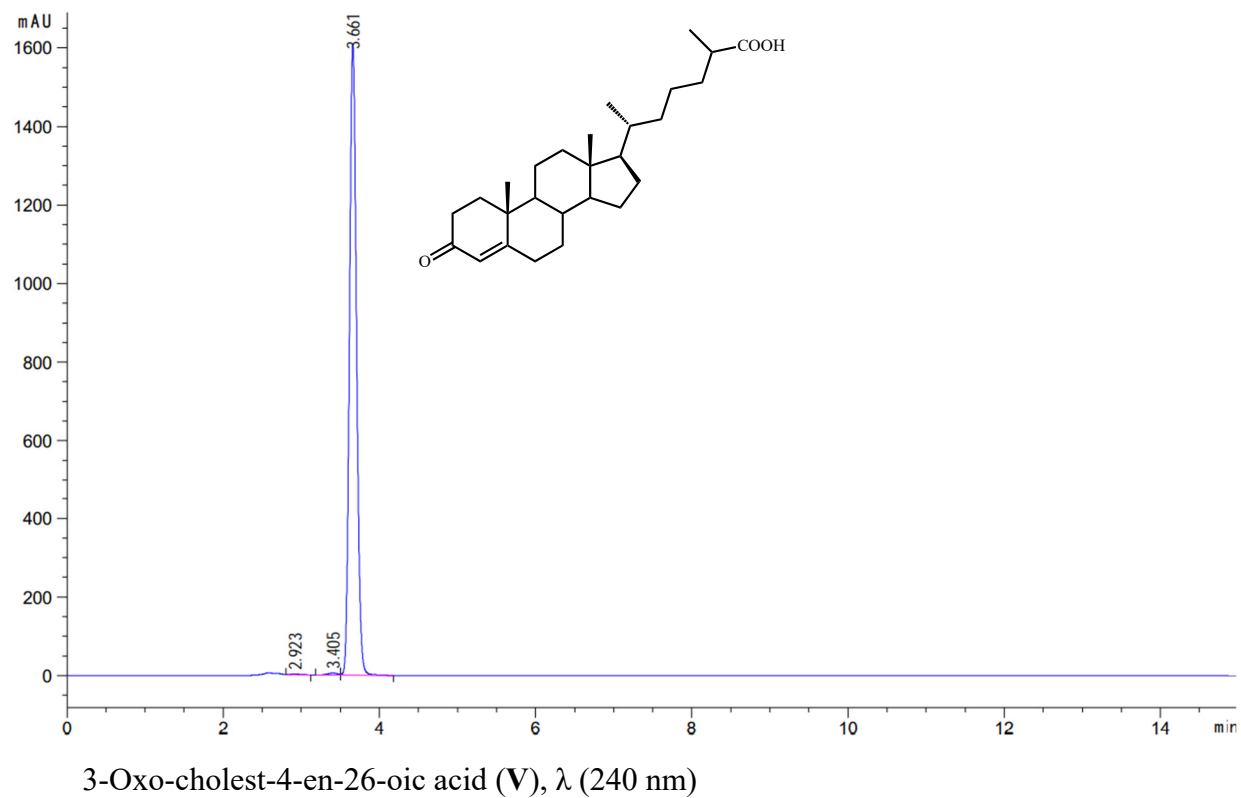




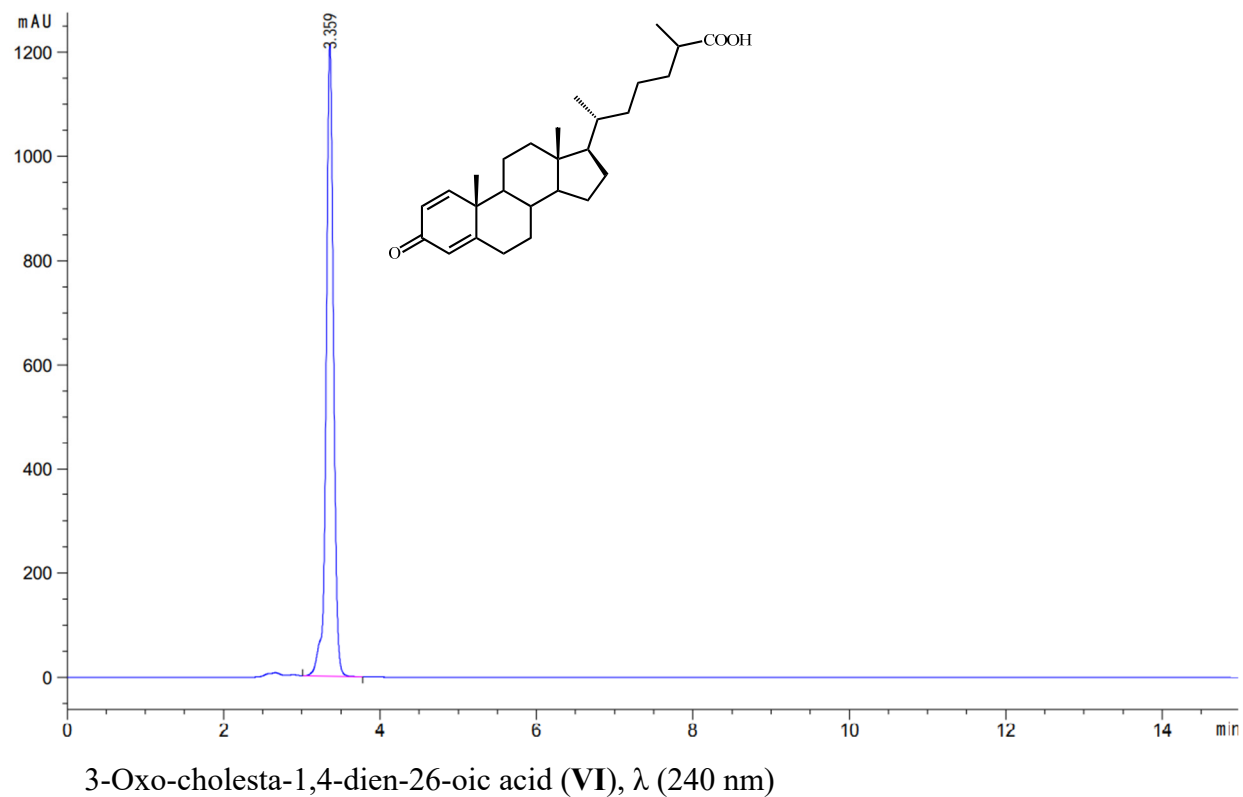
**Figure S5. HPLC-profile (mobile phase acetonitrile:2-propanol:water (50:45:5 v/v/v))  
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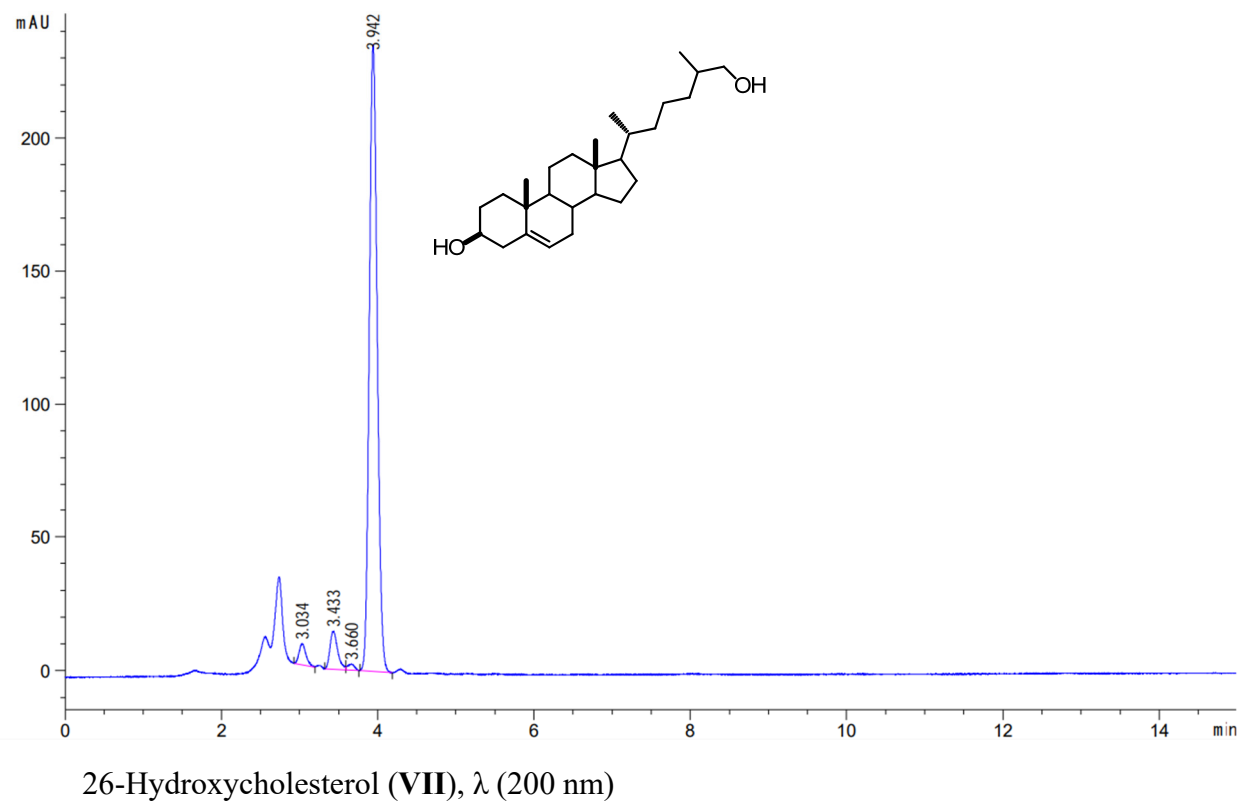
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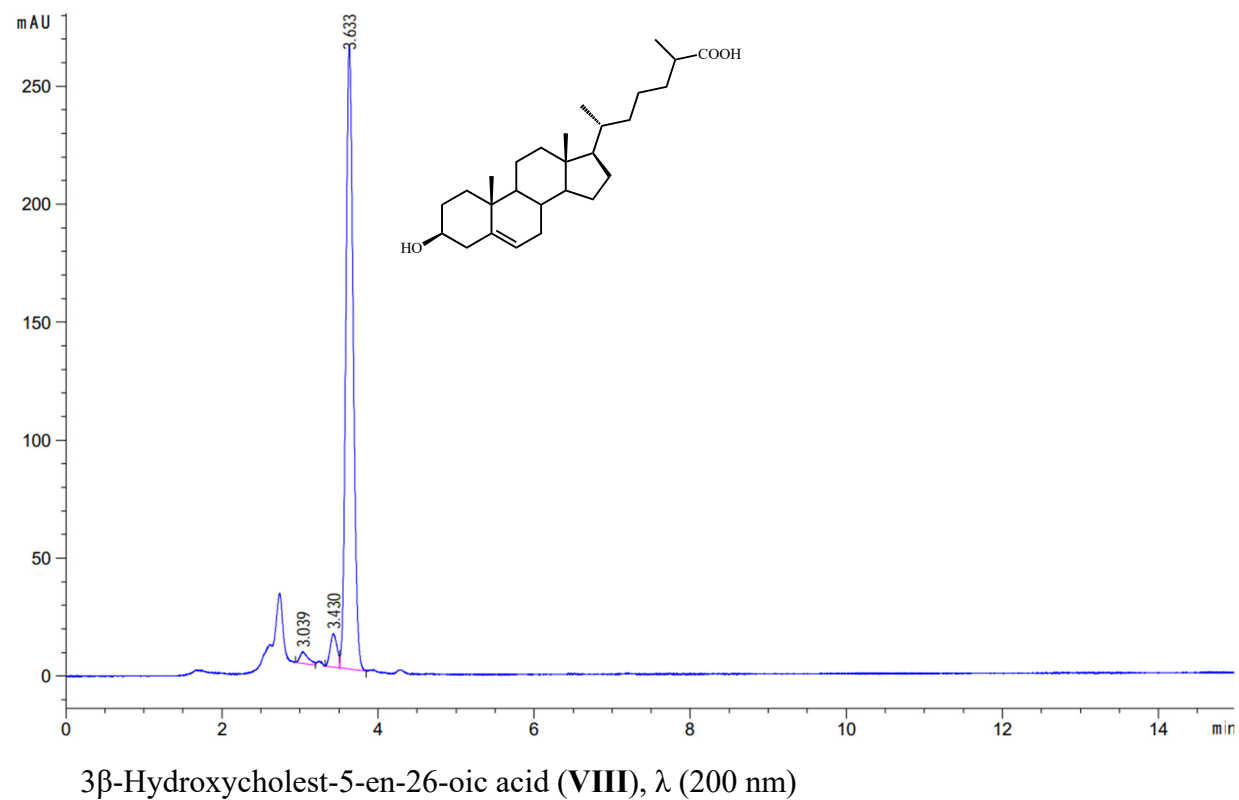
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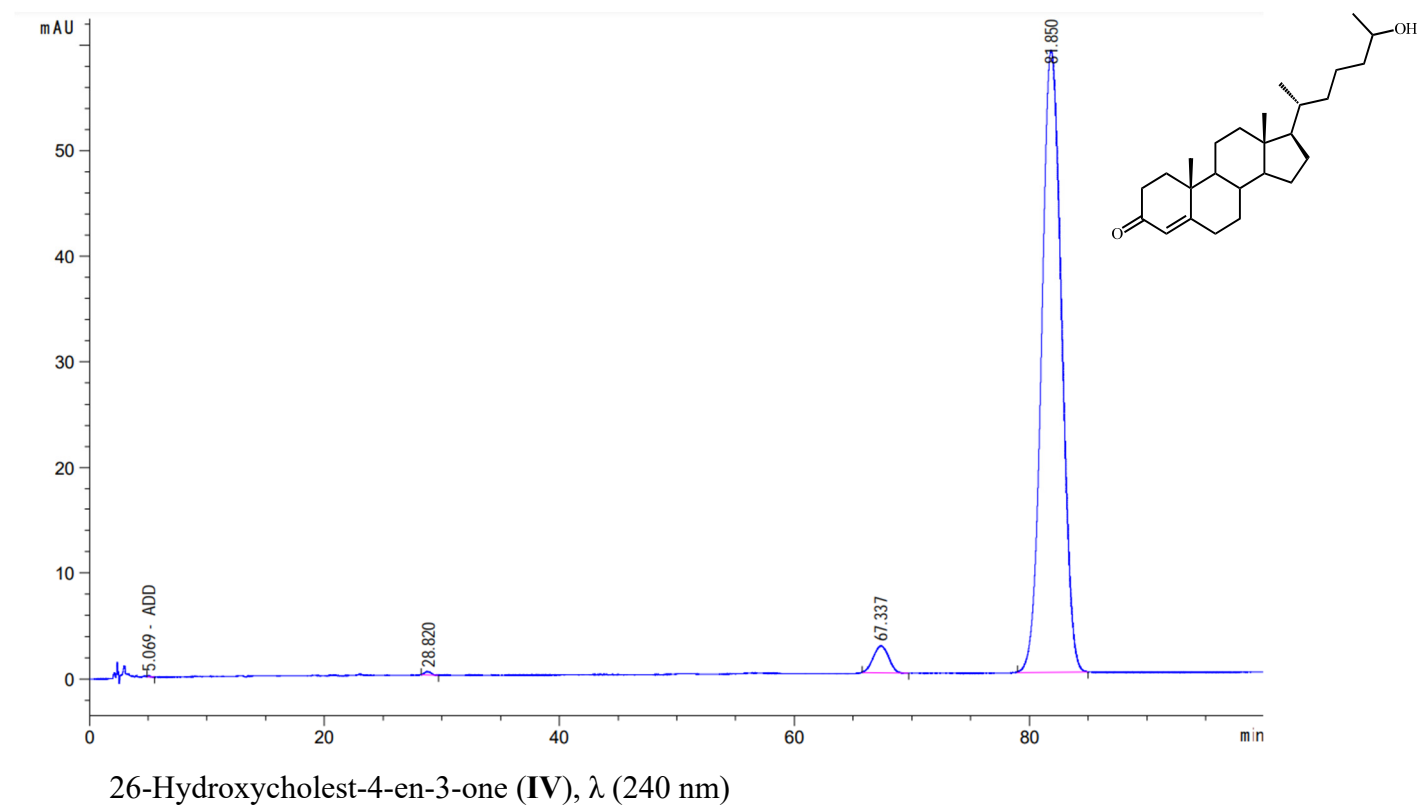
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of the individual compound isolated from the transformation media at the cholesterol conversion by *S.hirsuta***



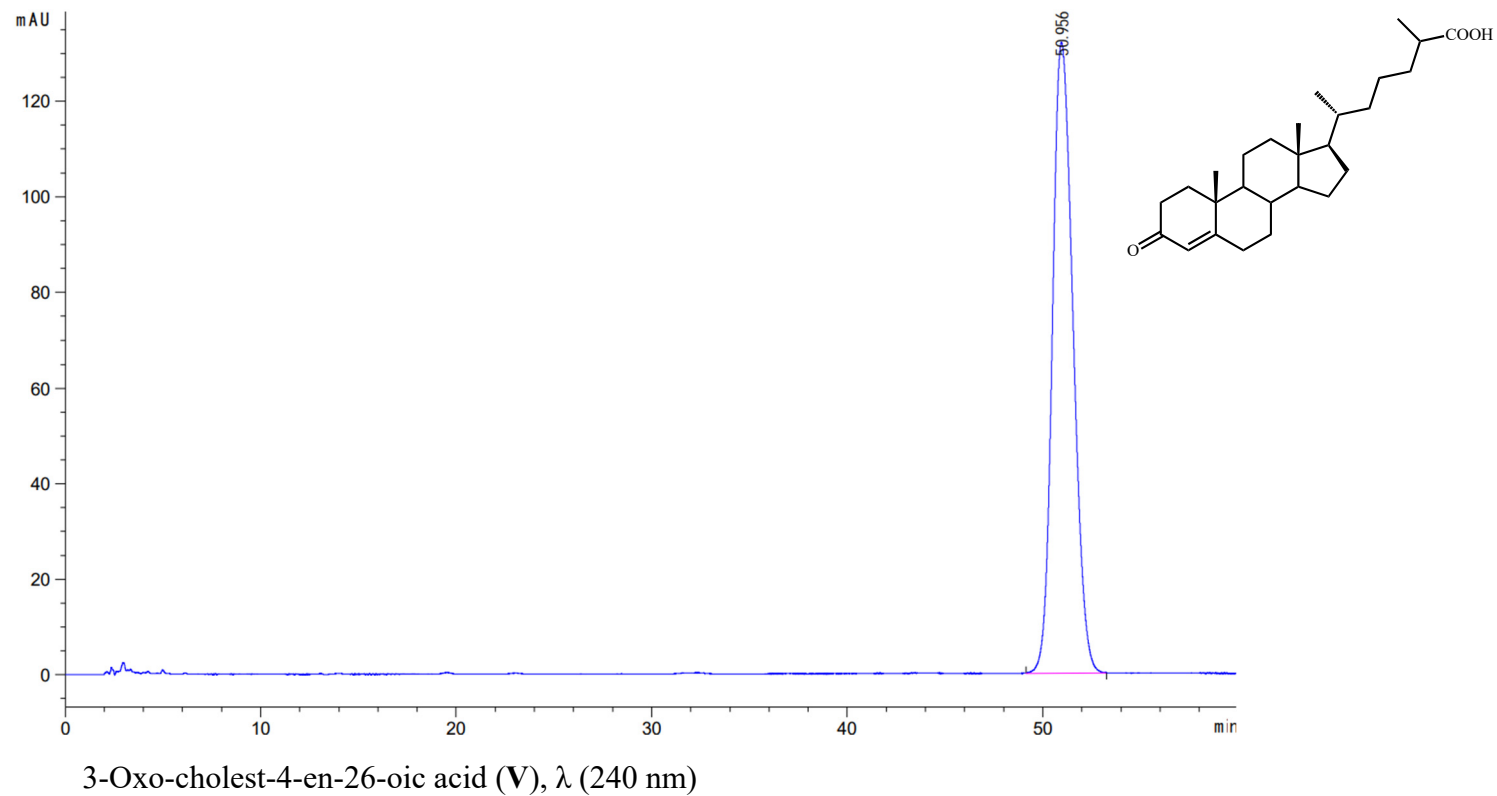
**Figure S9. HPLC-profile (mobile phase acetonitrile:2-propanol:water (50:45:5 v/v/v))  
of the individual compound isolated from the transformation media at the cholesterol conversion by *S.hirsuta***



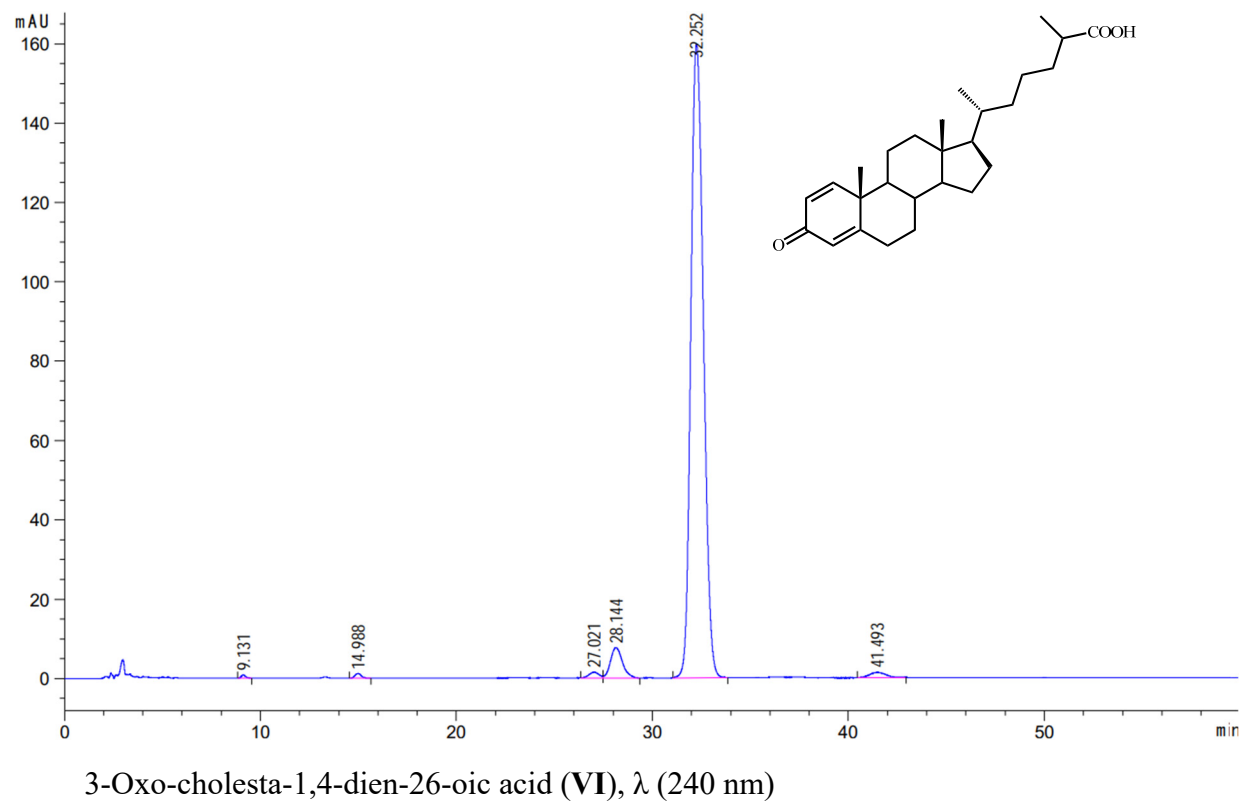
**Figure S10. HPLC-profile (mobile phase acetonitrile:water:acetic acid (60:40:0.01 v/v/v))  
of the individual compound isolated from the transformation media at the cholesterol conversion by *S.hirsuta***



**Figure S11. HPLC-profile (mobile phase acetonitrile:water:acetic acid (60:40:0.01 v/v/v/))  
of the individual compound isolated from the transformation media at the cholesterol conversion by *S.hirsuta***

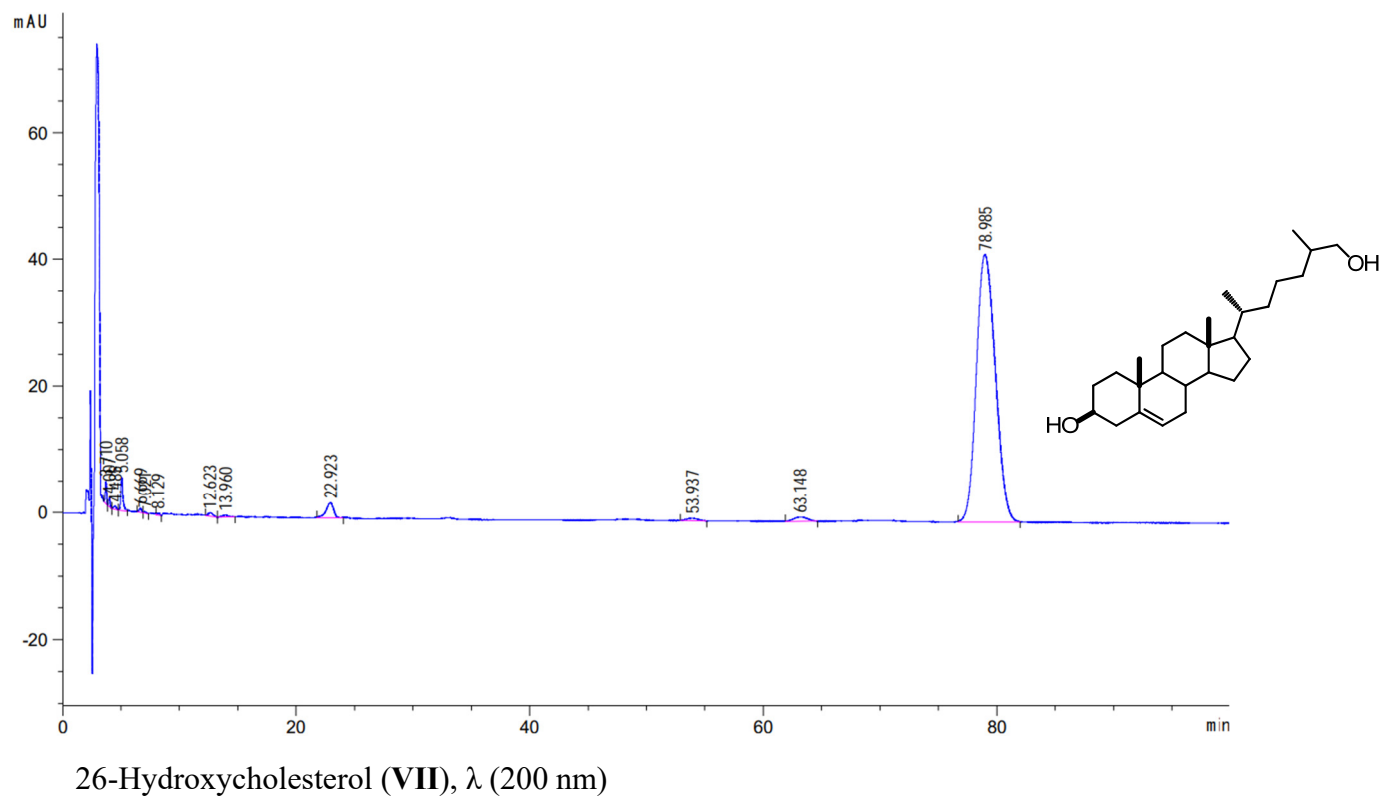


**Figure S12. HPLC-profile (mobile phase acetonitrile:water:acetic acid (60:40:0.01 v/v/v))  
of the individual compound isolated from the transformation media at the cholesterol conversion by *S.hirsuta***

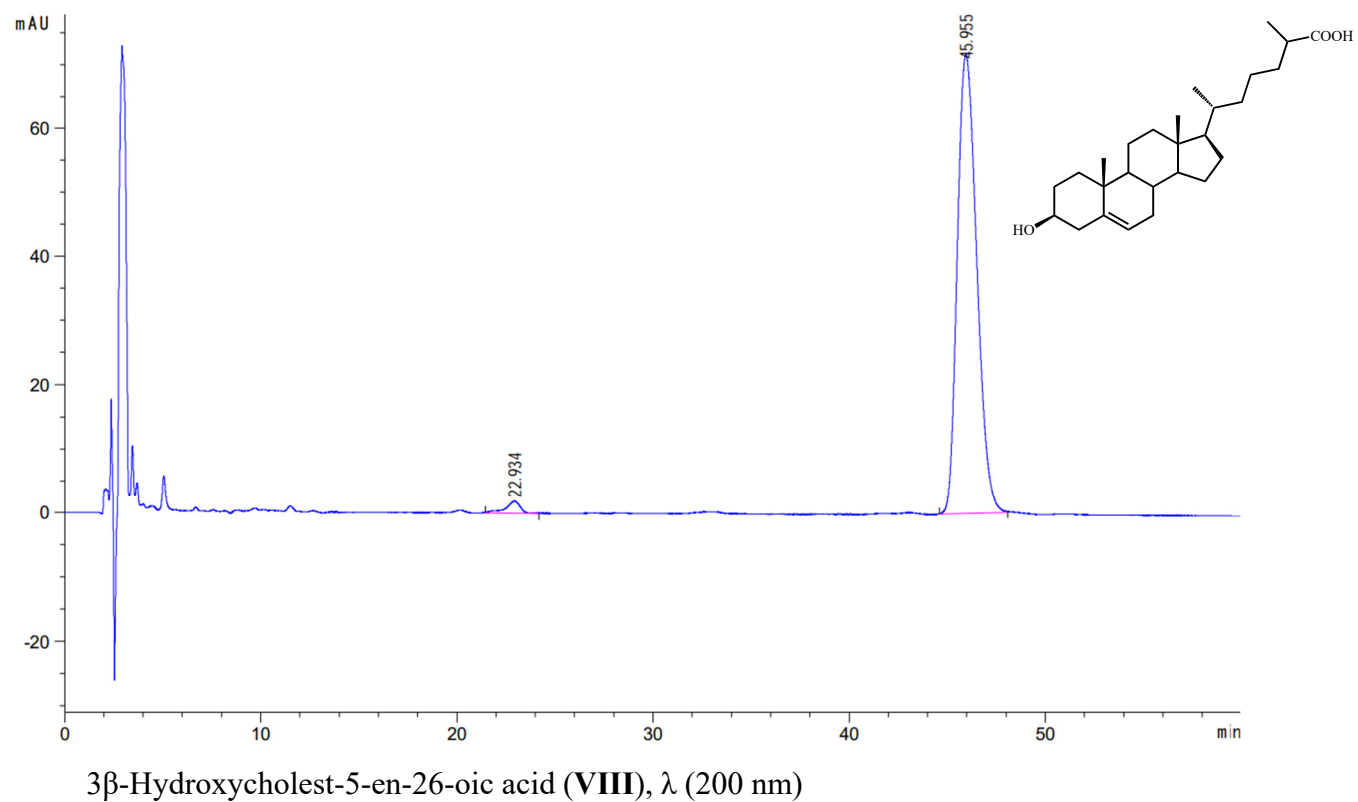




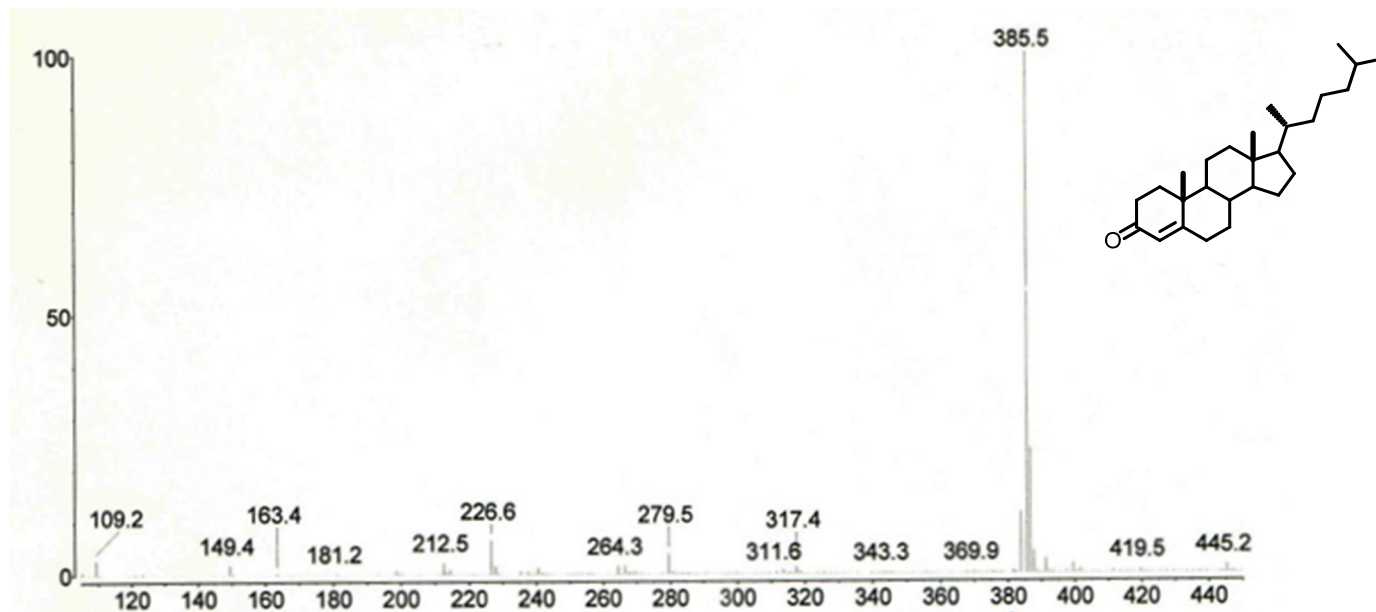
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**Figure S14. HPLC-profile (mobile phase acetonitrile:water:acetic acid (60:40:0.01 v/v/v))  
of the individual compound isolated from the transformation media at the cholesterol conversion by *S.hirsuta***

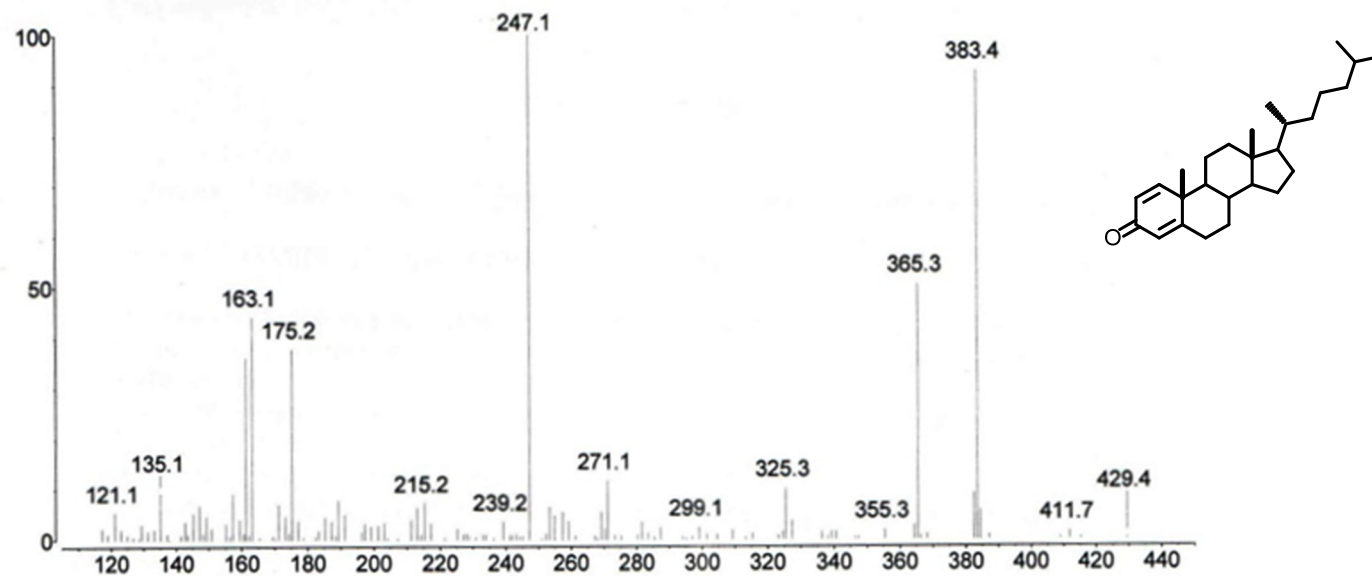


**Figure S15. Mass spectrum  $[M+H]^+$**



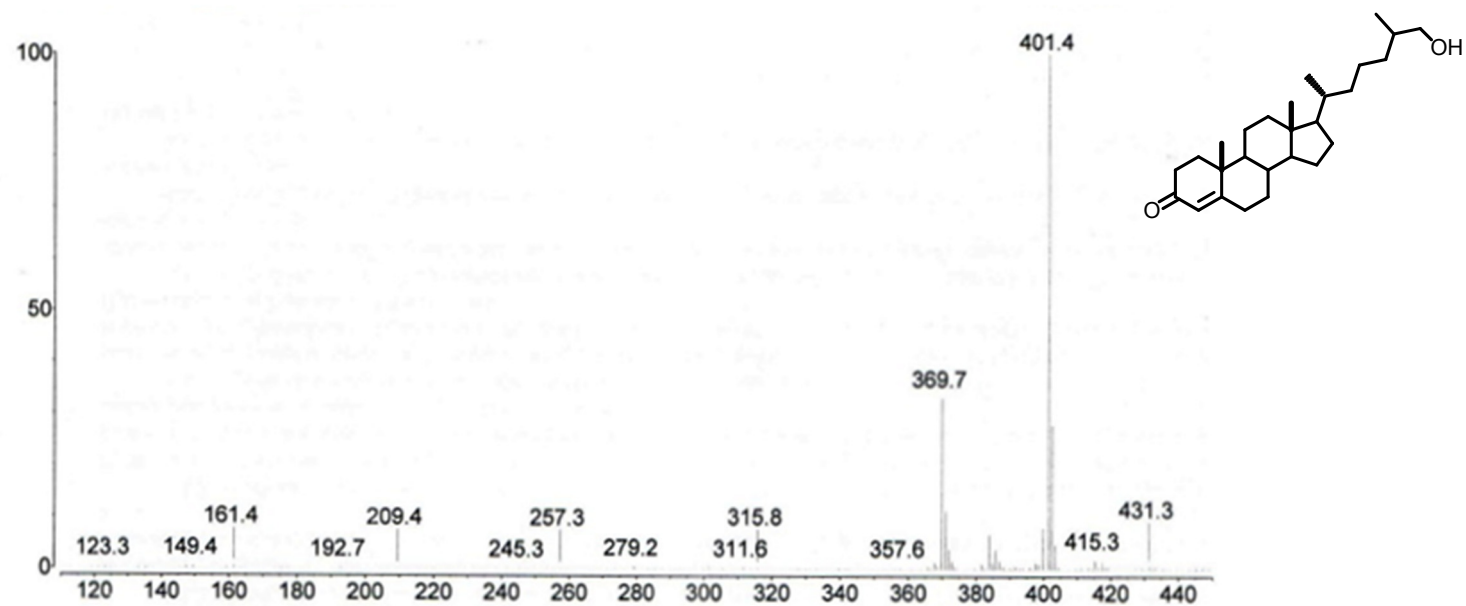
Cholest-4-en-3-one (384) (II)

**Figure S16. Mass spectrum  $[M+H]^+$**



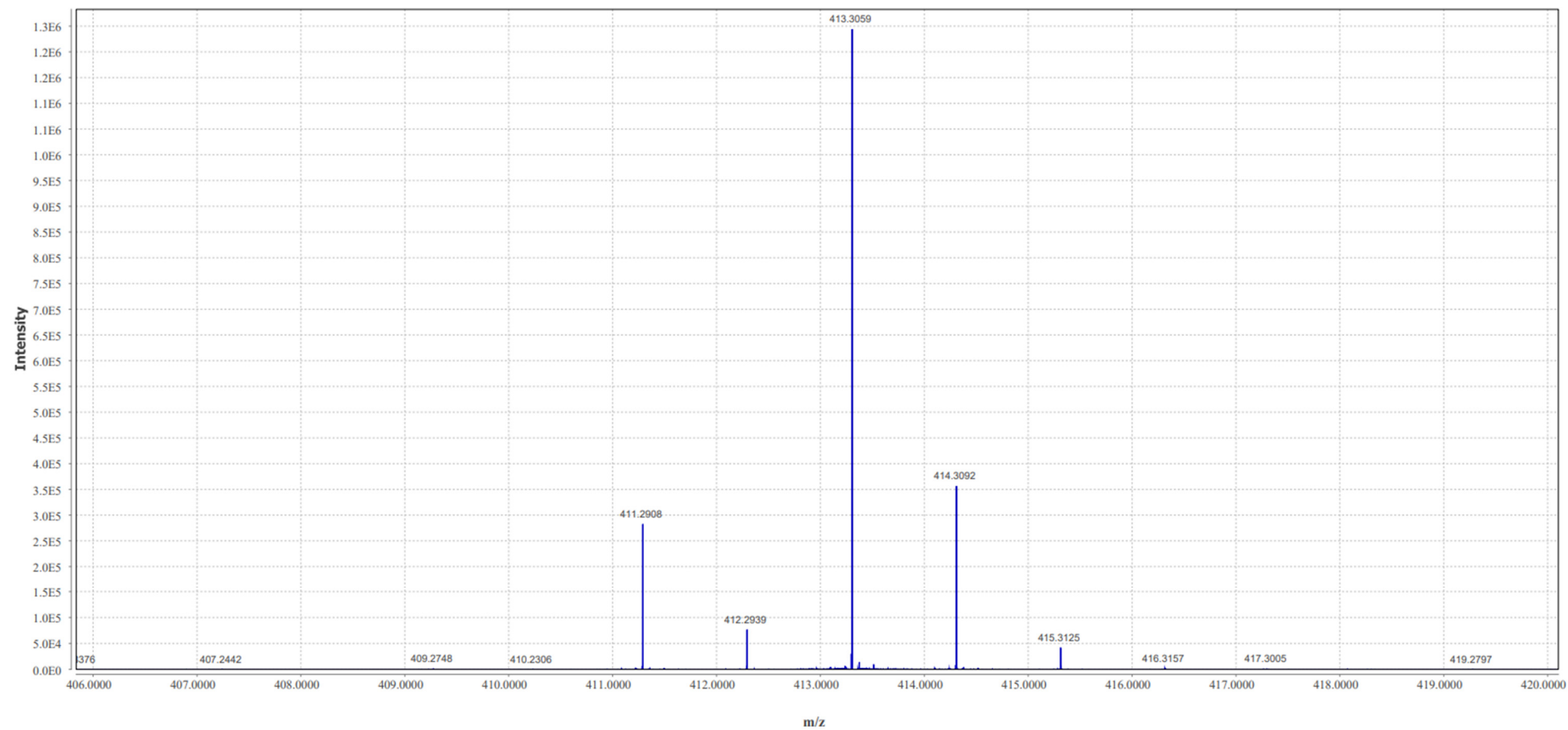
Cholesta-1,4-dien-3-one (**382**) (**III**), (collision energy 33 eV)

**Figure S17. Mass spectrum  $[M+H]^+$**



26-Hydroxy-cholest-4-en-3-one (**400**) (**IV**)

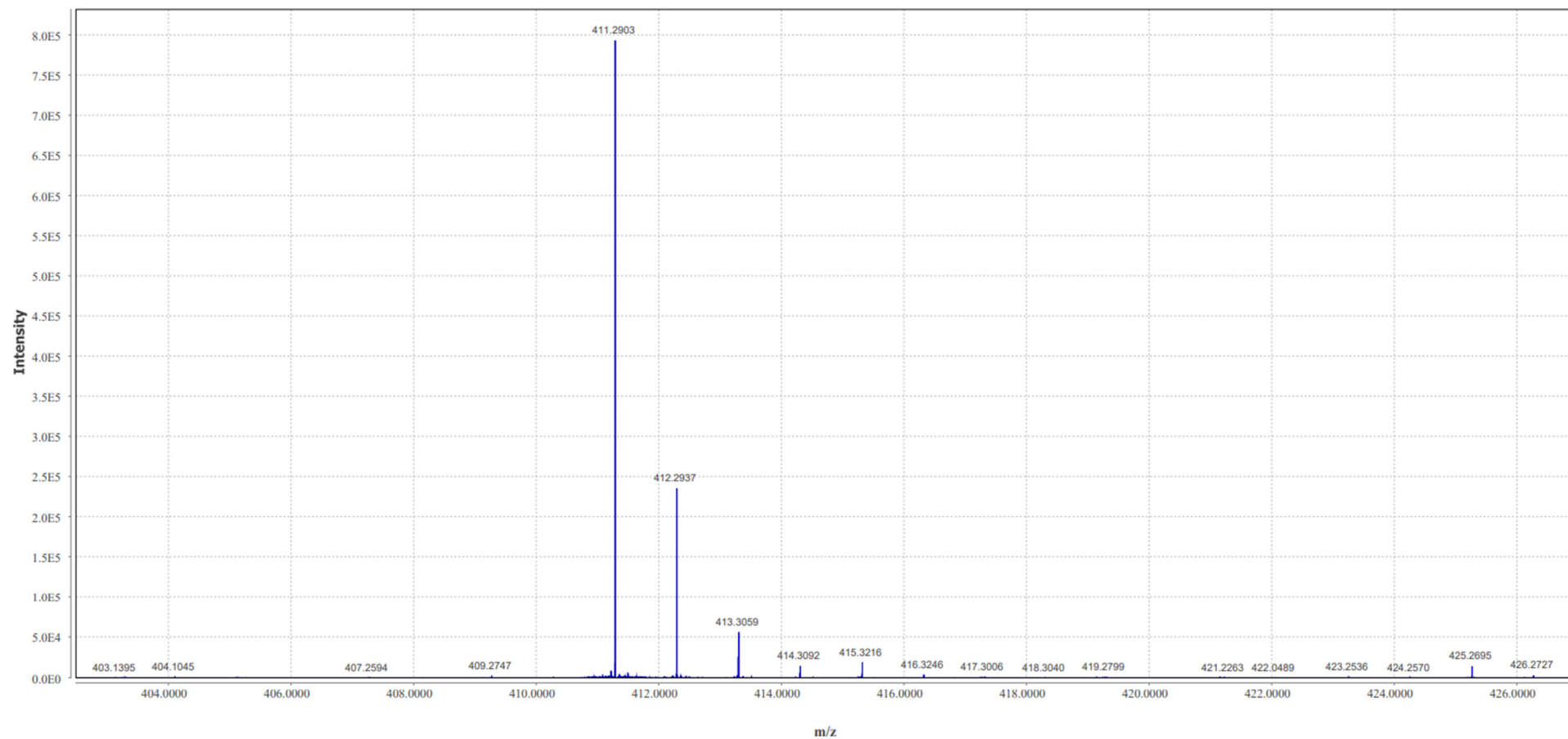
**Figure S18. Mass spectrum  $[M-H]^+$**



HRMS-ESI ( $m/z$ ):  $[M-H]^+$  calcd for  $C_{27}H_{41}O_3$  413,3056; found 413,3059

3-Oxo-cholest-4-en-26-oic acid (**414**) (V)

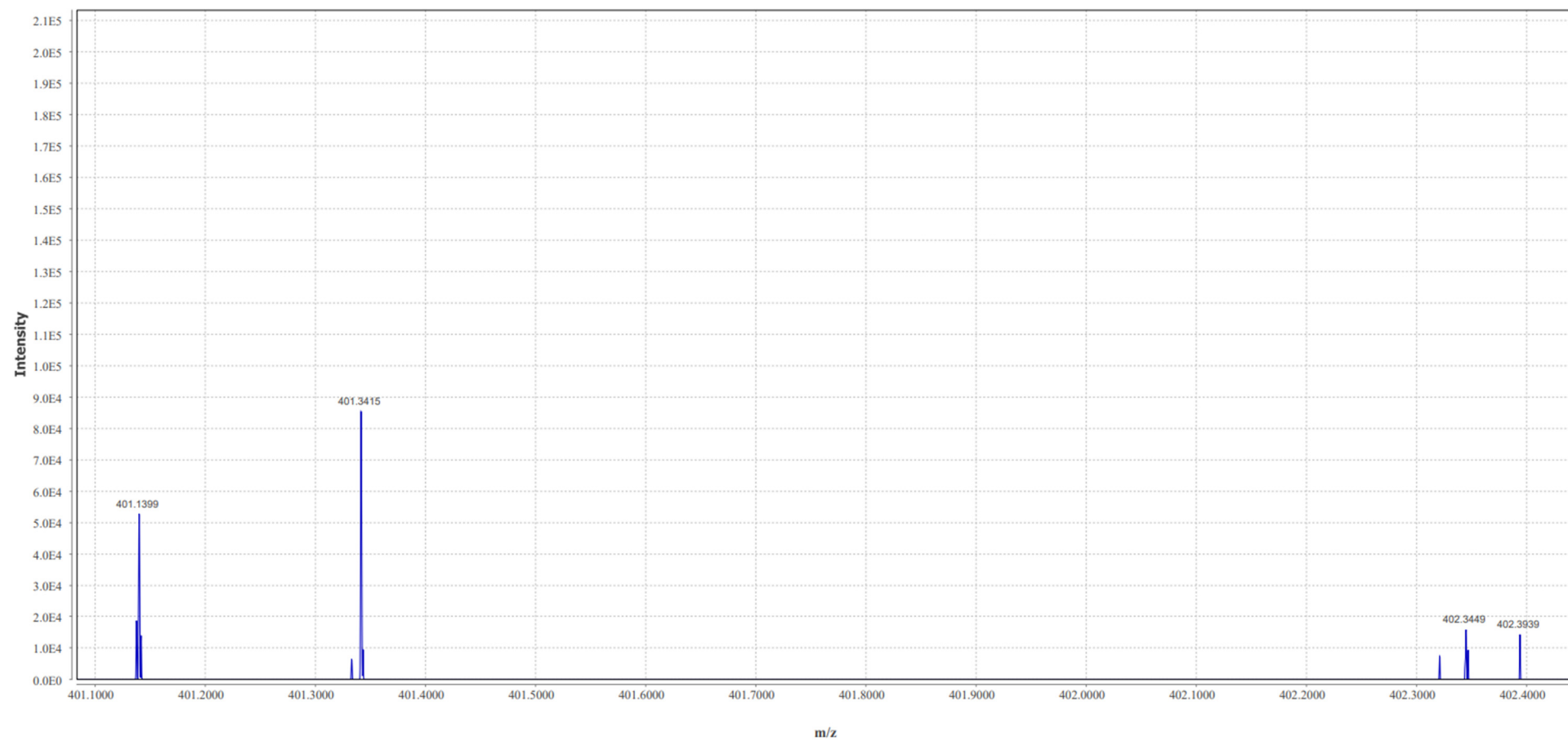
**Figure S19. Mass spectrum  $[M-H]^+$**



HRMS-ESI ( $m/z$ ):  $[M-H]^+$  calcd for  $C_{27}H_{39}O_3$  411,2899; found 411, 2903

3-Oxo-cholesta-1,4-dien-26-oic acid (**412**) (**VI**)

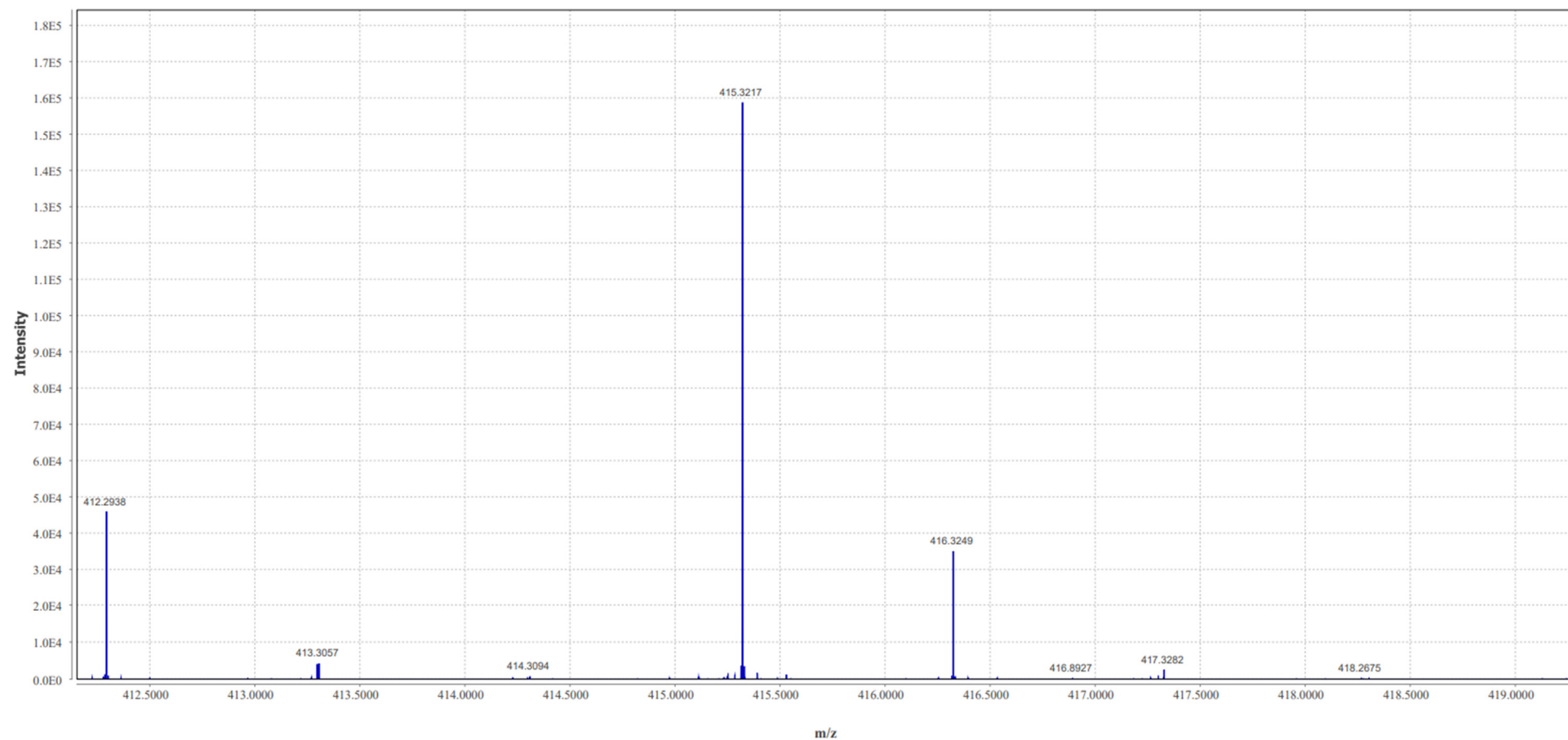
**Figure S20. Mass spectrum  $[M-H]^+$**



HRMS-ESI (m/z):  $[M-H]^+$  calcd for  $C_{27}H_{45}O_2$  401,3420; found 401,3415

26-Hydroxycholesterol (**402**) (**VII**)

**Figure S21. Mass spectrum  $[M-H]^+$**

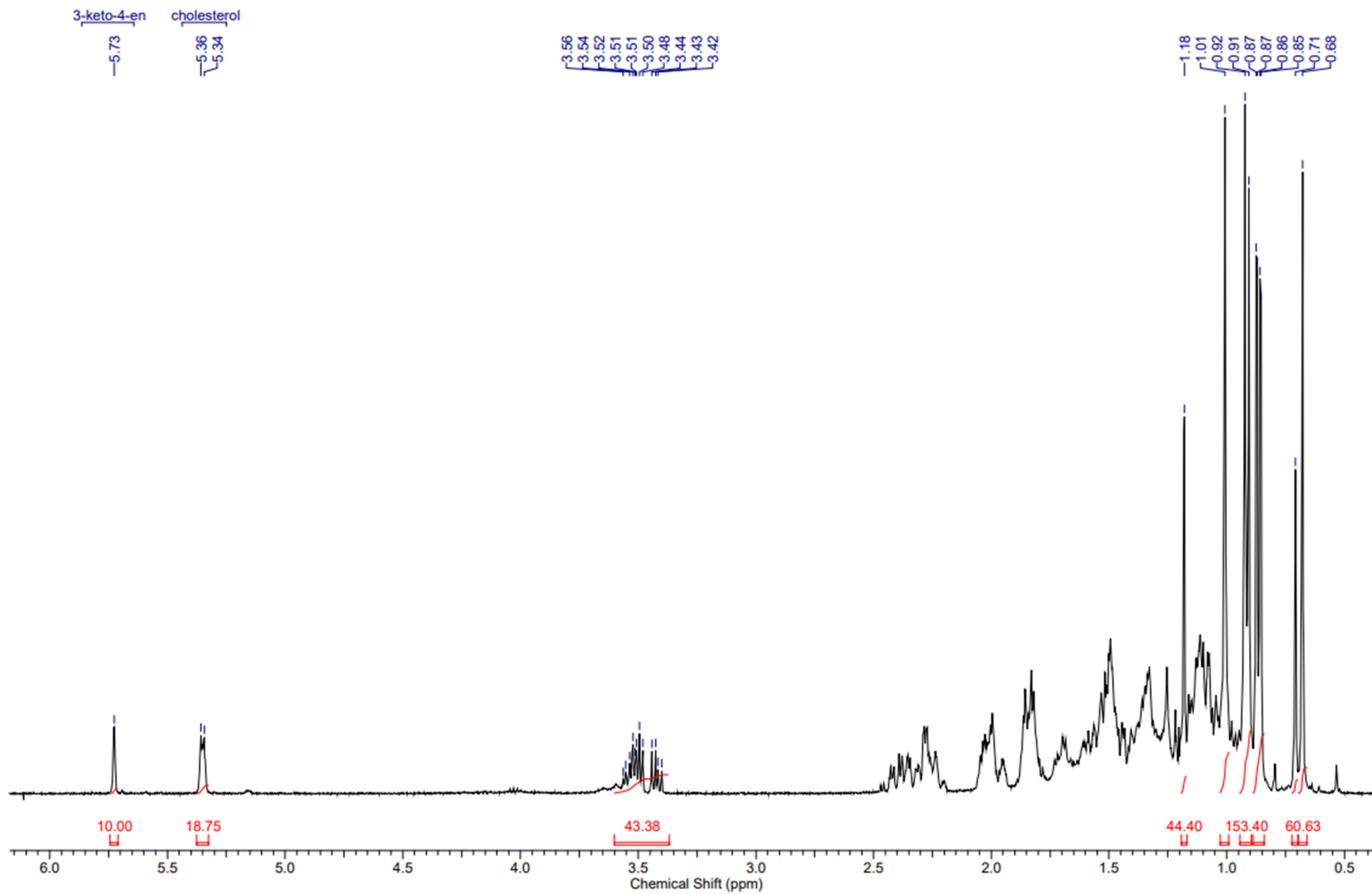


HRMS-ESI ( $m/z$ ):  $[M-H]^+$  calcd for  $C_{27}H_{43}O_3$  415,3212; found 415,3217

3 $\beta$ -Hydroxycholest-5-en-26-oic acid (**416**) (**VIII**)

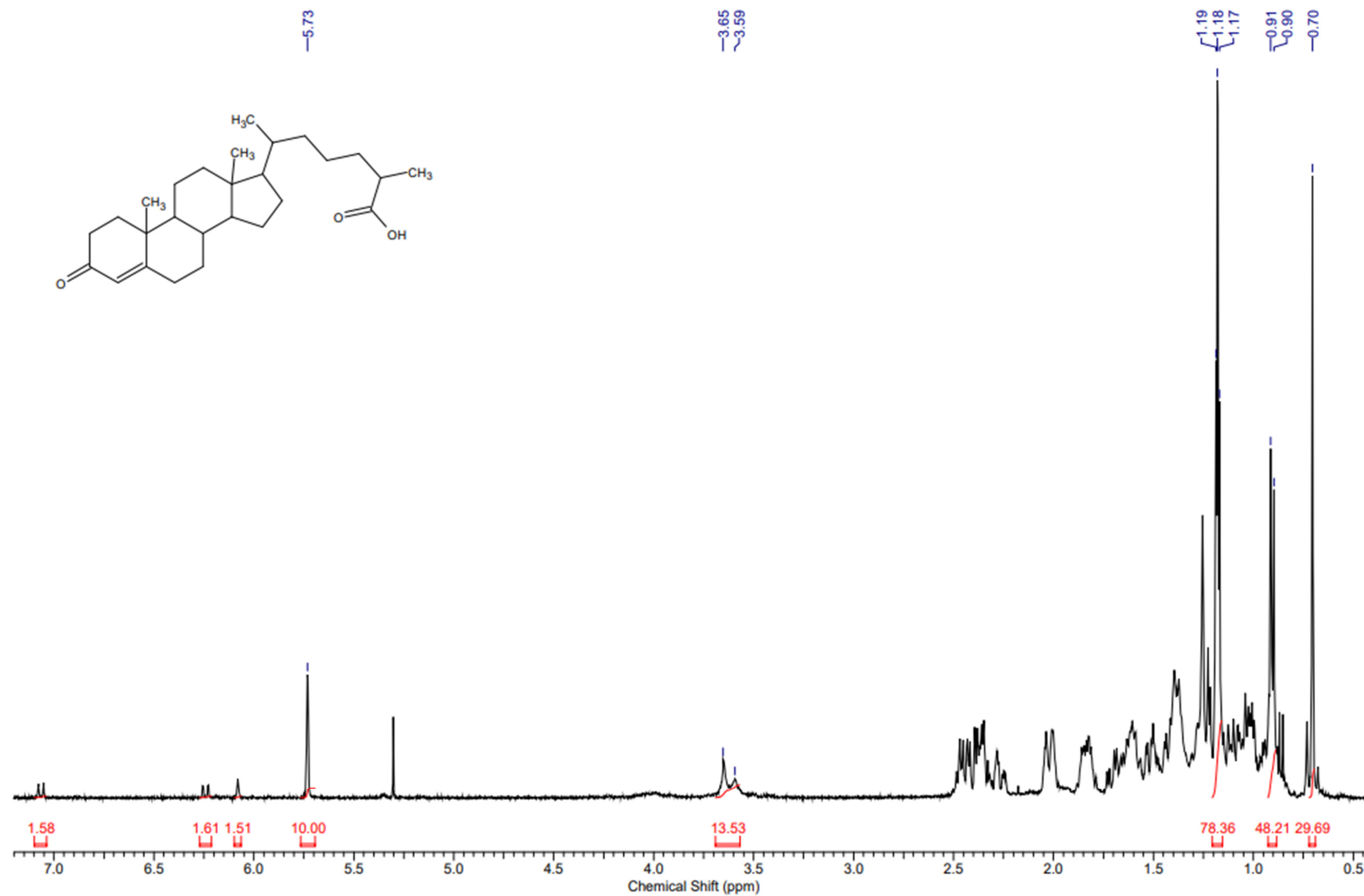


Figure S22.  $^1\text{H}$ -NMR spectrum



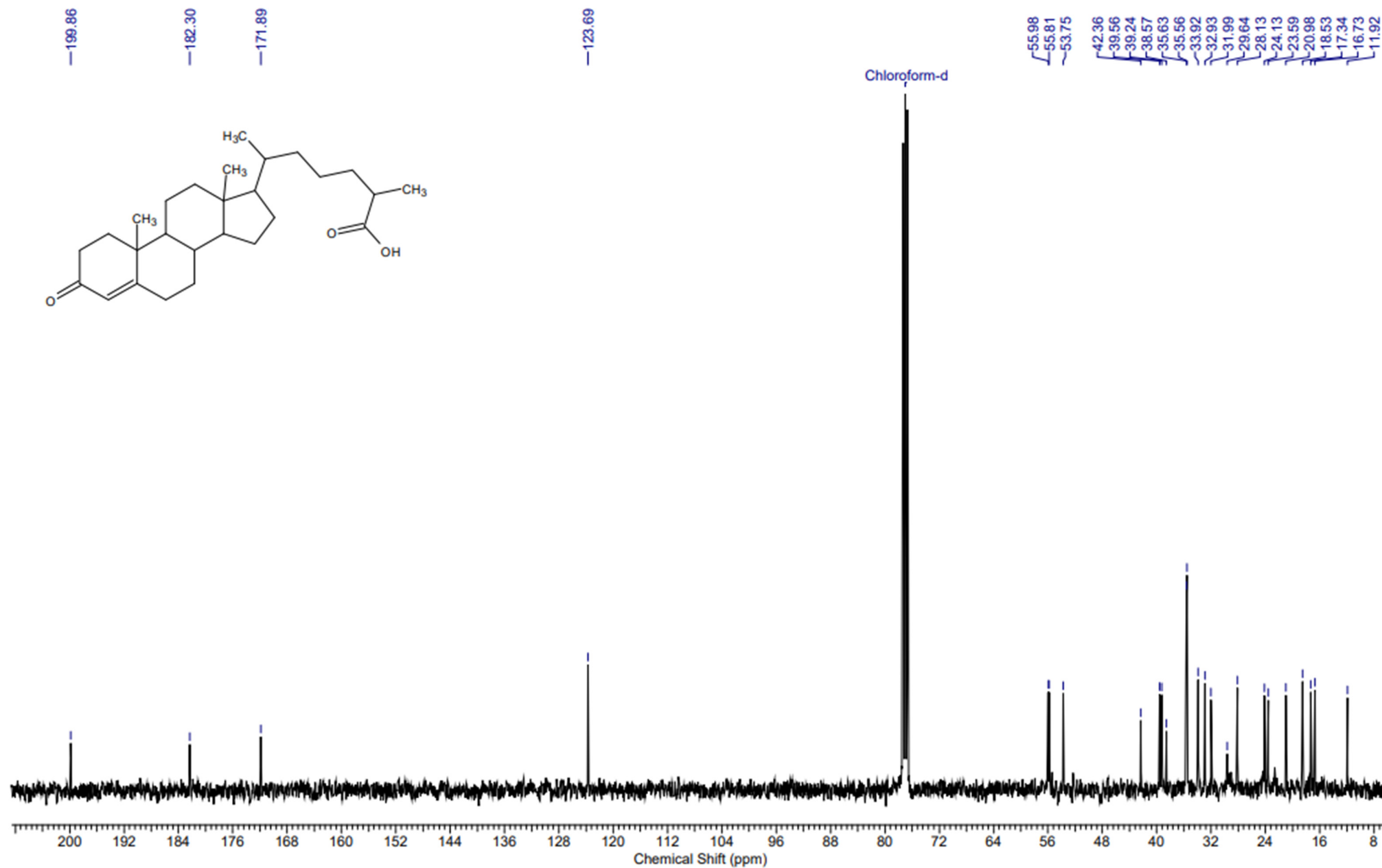
26-Hydroxycholest-4-en-3-one (IV)

Figure S23.  $^1\text{H}$ -NMR spectrum



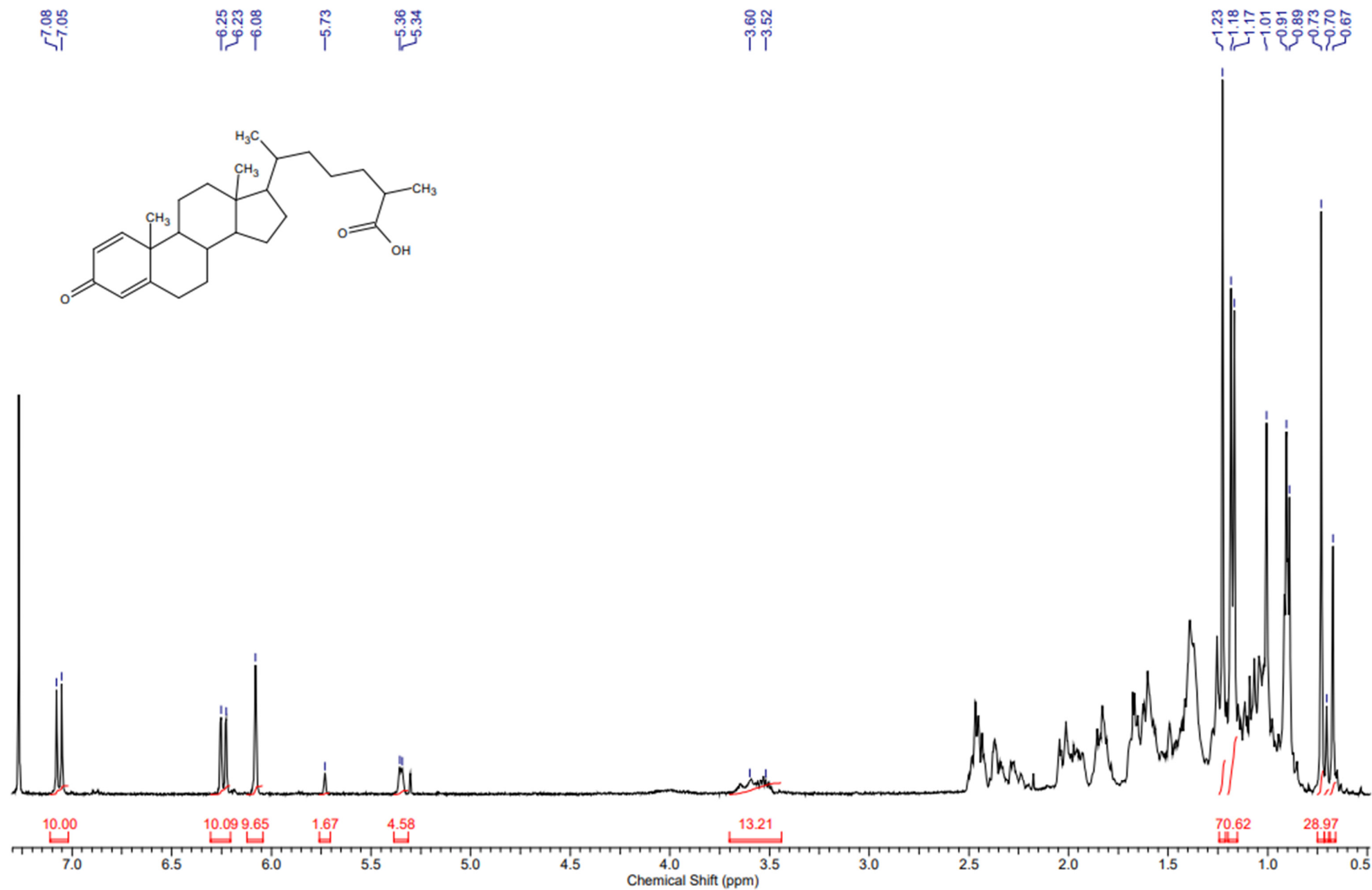
3-Oxo-cholest-4-en-26-oic acid (V)

Figure S24.  $^{13}\text{C}$ -NMR spectrum



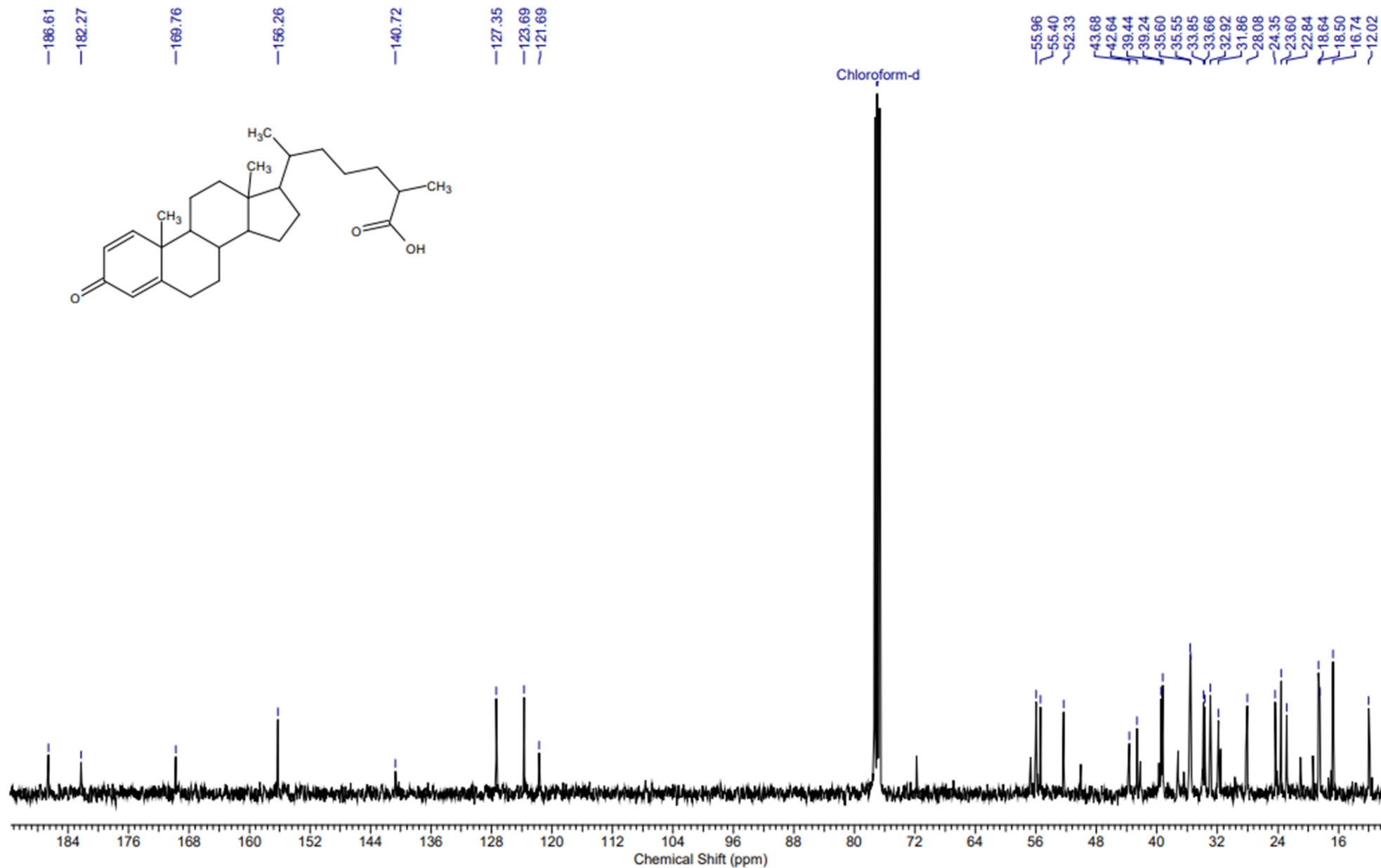
3-Oxo-cholest-4-en-26-oic acid (V)

Figure S25.  $^1\text{H}$ -NMR spectrum



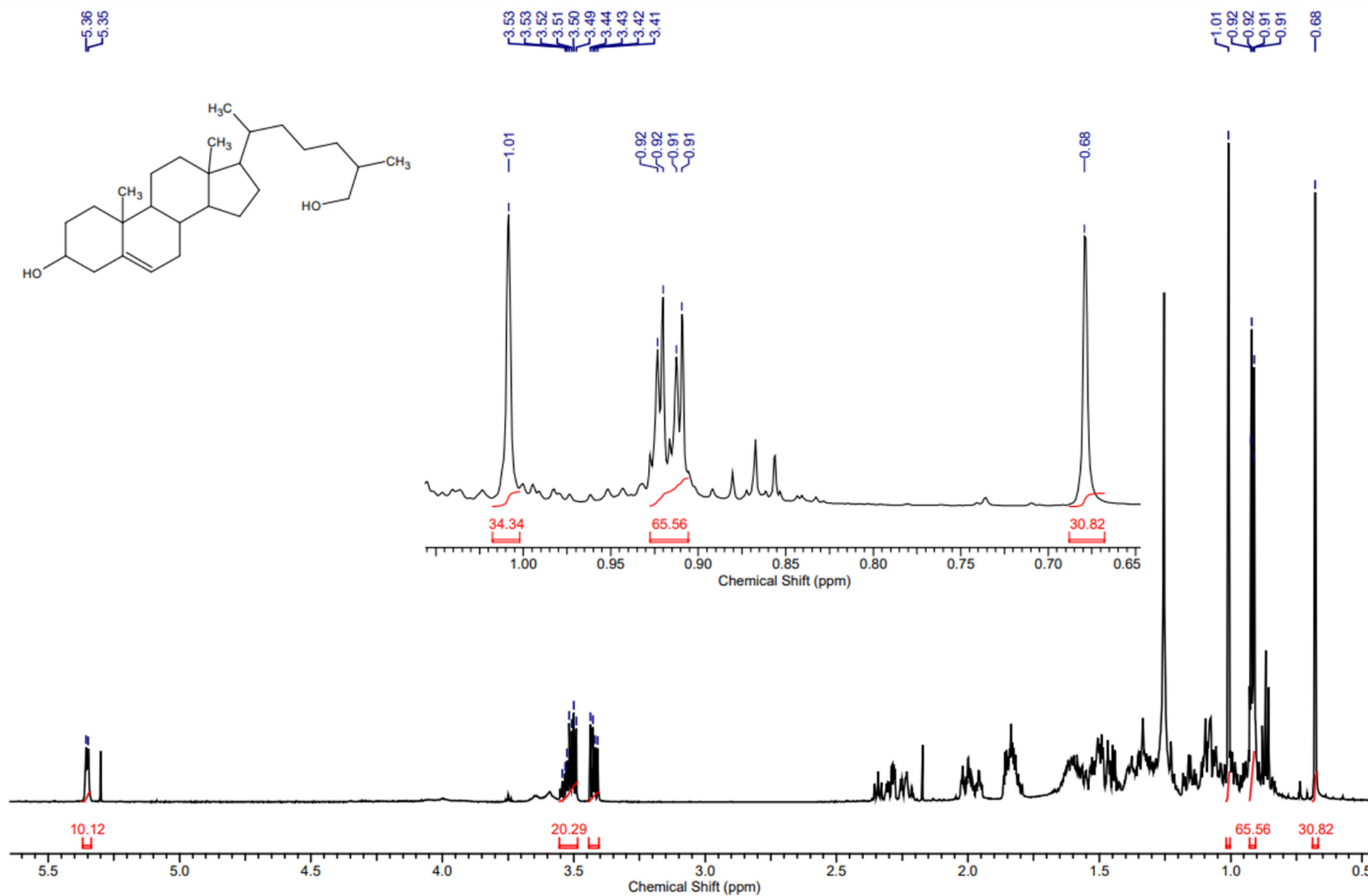
3-Oxo-cholesta-1,4-dien-26-oic acid (VI)

Figure S26.  $^{13}\text{C}$ -NMR spectrum



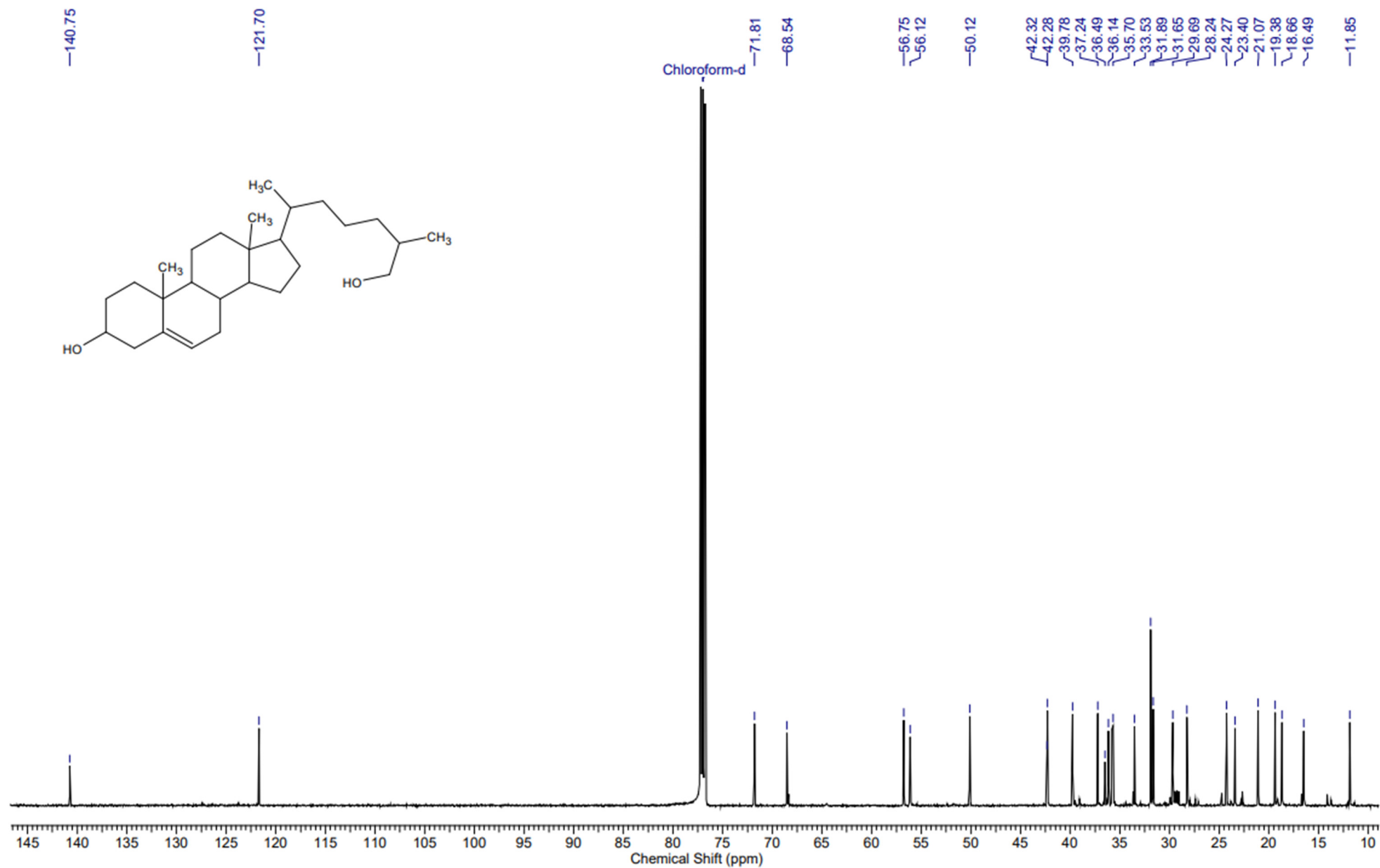
3-Oxo-cholesta-1,4-dien-26-oic acid (VI)

Figure S27.  $^1\text{H}$ -NMR spectrum



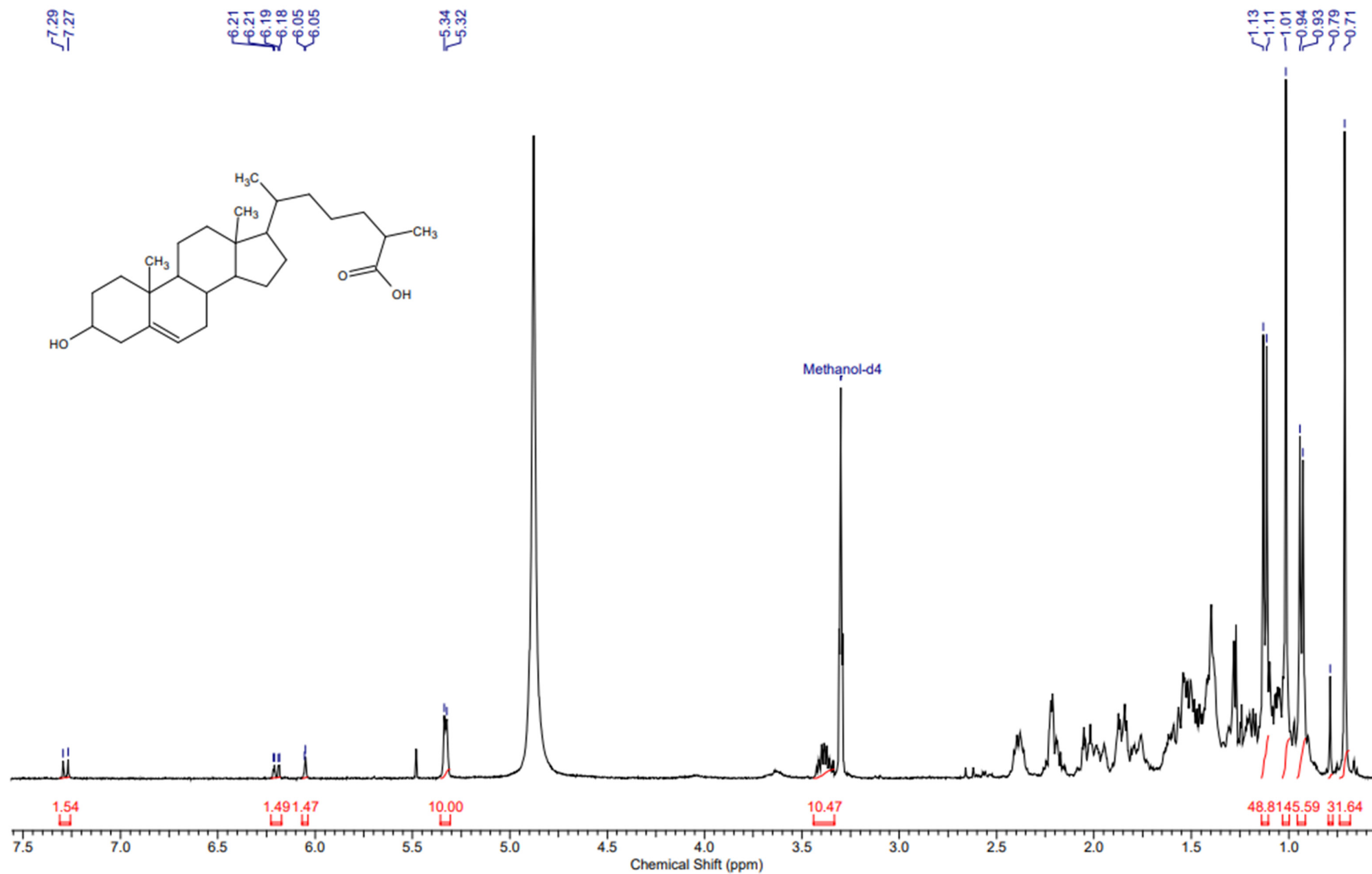
26-Hydroxycholesterol (VII)

Figure S28.  $^{13}\text{C}$ -NMR spectrum



26-Hydroxycholesterol (VII)

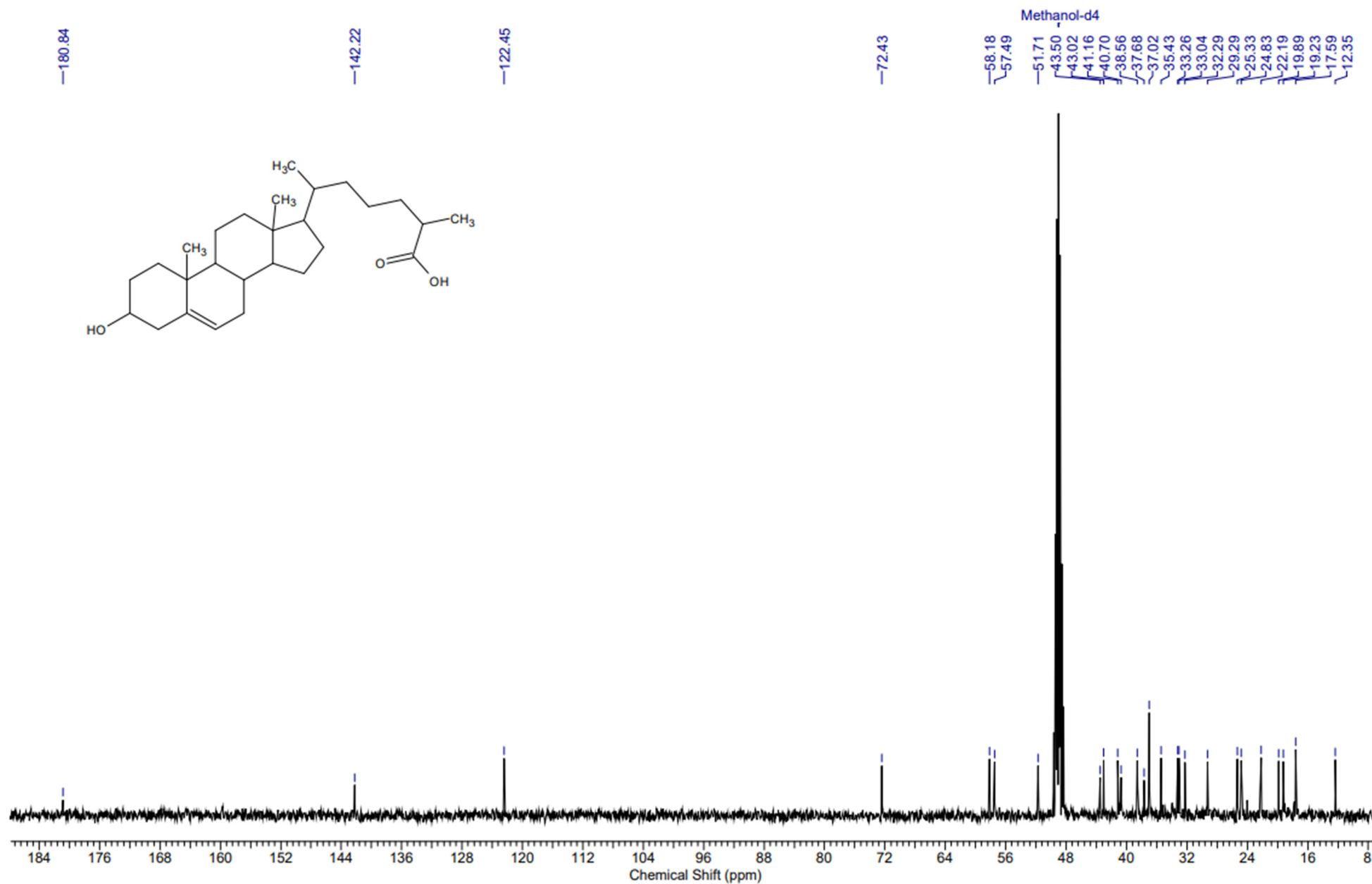
Figure S29.  $^1\text{H}$ -NMR spectrum



3β-Hydroxycholest-5-en-26-oic acid (VIII)

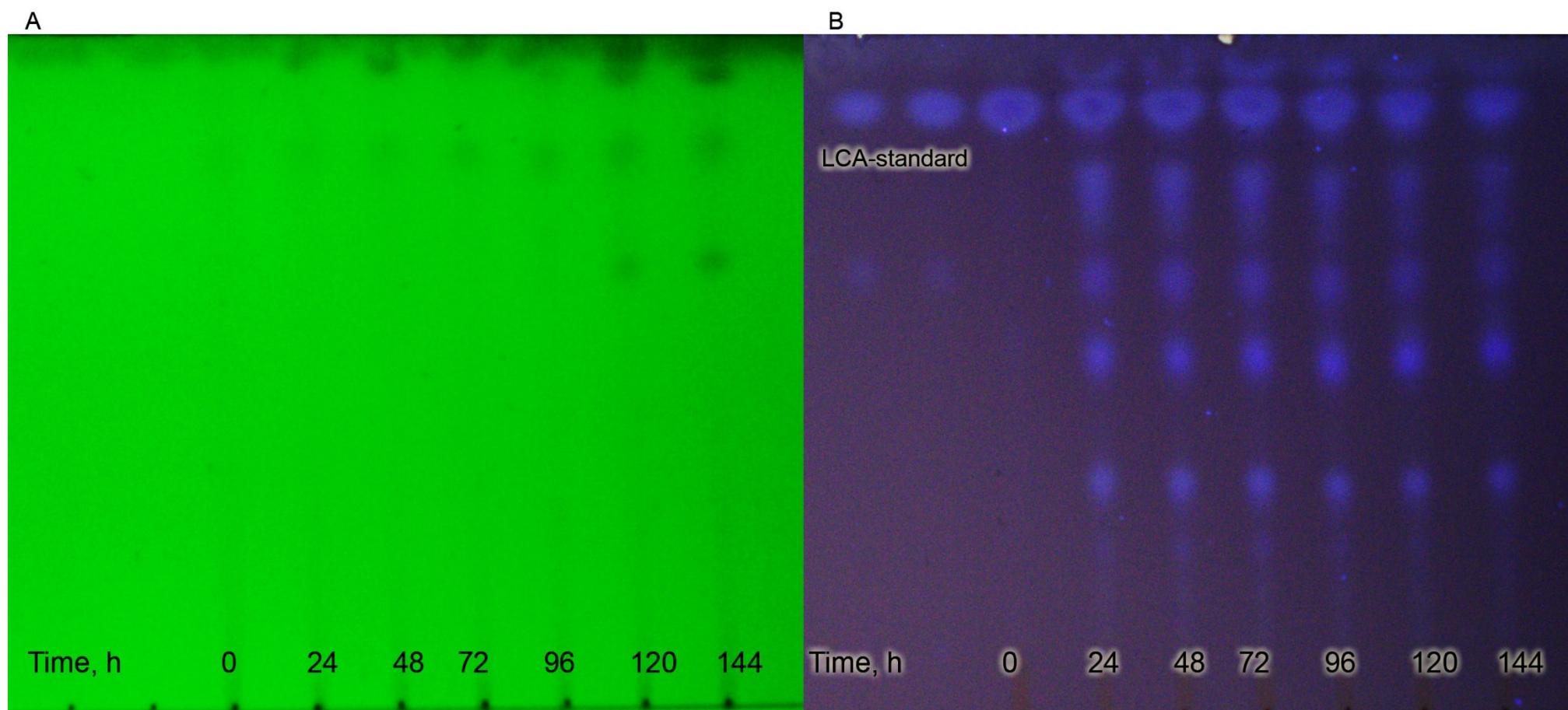


Figure S30.  $^{13}\text{C}$ -NMR spectrum



3β-Hydroxycholest-5-en-26-oic acid (VIII)

**Figure S31. Bioconversion of lithocholic acid by *S.hirsuta* VKM Ac-666<sup>T</sup> under the conditions used**



**A.** 3-Keto-4-ene steroids, system CHCl<sub>3</sub>/acetone/CH<sub>3</sub>COOH (50:50:0.5), visualization by UV light (254 nm)

**B.** Compounds with the unchanged A-ring structure, system CHCl<sub>3</sub>/acetone/CH<sub>3</sub>COOH (50:50:0.5); staining of TLC plates using MnCl<sub>2</sub> solution, visualization by UV light (365 nm), reference compound – lithocholic acid (LCA)