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New lupane triterpenoids from *Solidago canadensis* that inhibit the lyase activity of DNA polymerase β

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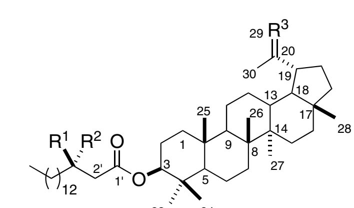
Abstract—Bioassay-directed fractionation of a methyl ethyl ketone extract of *Solidago canadensis* L. (Asteraceae), using an assay to detect the lyase activity of DNA polymerase β, resulted in the isolation of the four new lupane triterpenoids **1**–**4** and the seven known compounds lupeol, lupeyl acetate, ursolic acid, cycloartenol, cycloartenyl palmitate, α-amyrin acetate, and stigmasterol. The structures of the new compounds were established as 3β-(3*R*-acetoxyhexadecanoyloxy)-lup-20(29)-ene (**1**), 3β-(3-ketohexadecanoyloxy)-lup-20(29)-ene (**2**), 3β-(3*R*-acetoxyhexadecanoyloxy)-29-*nor*-lupan-20-one (**3**), and 3β-(3-ketohexadecanoyloxy)-29-*nor*-lupan-20-one (**4**), respectively, on the basis of extensive 1D and 2D NMR spectroscopic interpretation and chemical modification studies. All 11 compounds were inhibitory to the lyase activity of DNA polymerase β.

1. Introduction

As part of our continuing efforts to identify novel naturally occurring anticancer agents,[1] an MEK extract of *Solidago canadensis* L. (Asteraceae) was found to show inhibitory activity toward the lyase activity of DNA polymerase β, and was selected for bioassay-guided fractionation using this assay. Previous phytochemical studies of *S. canadensis* resulted in the isolation of sesquiterpenes,[2] diterpenes,[3] saponins,[4] and flavonoids.[5] Initial liquid–liquid partition of the crude extract of *S. canadensis* indicated that the activity was equally distributed between the hexane and $CHCl_3$ fractions of hexane/aqueous MeOH and $CHCl_3$/aqueous MeOH partitions, respectively. The hexane and $CHCl_3$ residues were combined on the basis of their similar ^1H NMR spectra and TLC patterns. The combined residue was purified by chromatography over CHP2OP MCI gel (a highly porous polystyrene gel) followed by reversed-phase preparative TLC and HPLC to yield the four new lupane triterpenoids 3β-(3*R*-acetoxyhexadecanoyloxy)-lup-20(29)-ene (**1**), 3β-(3-ketohexadecanoyloxy)-lup-20(29)-ene (**2**), 3β-(3*R*-acetoxyhexadecanoyloxy)-29-*nor*-lupan-20-one (**3**), and 3β-(3-ketohexadecanoyloxy)-29-*nor*-lupan-20-one (**4**), in addition to seven known compounds. The latter were identified as lupeol,[6] lupeyl acetate, α-amyrin acetate,[7] ursolic acid, cycloartenol, cycloartenyl palmitate,[9] and stigmasterol[10] by comparison of their physical and spectroscopic data with literature data.



1 R^1 = $OCOCH_3$; R^2 = H; R^3 = CH_2
2 R^1 = R^2 = O; R^3 = CH_2
3 R^1 = $OCOCH_3$; R^2 = H; R^3 = O
4 R^1 = R^2 = R^3 = O
5 R^1 = OH; R^2 = H; R^3 = CH_2

2. Results and discussion

The molecular formula of **1** was deduced as $C_{48}H_{82}O_4$ by HRFABMS, ^{13}C NMR, and APT (attached proton test) spectra. It gave a positive Liebermann–Burchard (LB) test for triterpenoids.[11] The IR spectrum of **1**

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showed the presence of two carbonyl groups (1732 and 1723 cm^{-1}). The ^1H NMR spectrum of **1** showed the presence of six methyl singlets at δ 0.78 (3H), 0.82 ($2 \times 3H$), 0.84 (3H), 0.93 (3H), 1.02 (3H), an oxymethine group at δ 4.47 as a doublet of doublets ($J = 10.7$, 5.5 Hz), and a 2-propenyl group as inferred by the presence of a methyl singlet at δ 1.67 and peaks at δ 4.55 (d, $J = 2.5$ Hz) and 4.67 (d, $J = 2.5$ Hz). The two sp^2 carbons observed at δ 151.0 and 109.5 in the ^{13}C NMR spectrum of **1** supported the presence of the 2-propenyl group.[6] The ^1H NMR spectrum also showed the presence of an oxymethine proton as a multiplet centered at δ 5.18, an acetyl methyl singlet at δ 2.00, a methyl triplet at δ 0.87 ($J = 6.7$ Hz) and 13 methylene groups between δ 1.27 and 2.58 (Table 1).

The ^{13}C NMR values for all the carbons in **1** were assigned on the basis of APT, HMQC, and HMBC spectra and are given in Table 2. The ^1H NMR spectrum, especially the presence of a 2-propenyl group, suggested that compound **1** is a pentacyclic triterpene of the lup-20(29)-ene-3β-ol or hop-22(29)-3β-ol type. The basic skeleton of a hop-22(29)-3β-ol triterpenoid could be ruled out for compound **1** on the basis of the differences in the ^{13}C NMR values of **1** with those of hopenol derivatives.[6] On the other hand, the ^1H and ^{13}C NMR values of **1** were almost identical to those of 3β-(3*R*-hydroxyhexadecanoyloxy)-lup-20(29)-ene (**5**) isolated from *Maclura pomifera* (Raf.) C.K.Schneid (Moraceae),[12] except for the presence of additional signals in **1** corresponding to an acetyl group. The basic skeleton of **1** was supported by the key HMBC correlations: H-3/C-1, C-2, C-4, C-5, C-23, C-24, C-1′; H-6/C-4, C-5, C-7, C-8, C-10; H-9/C-1, C-5, C-7, C-8, C-10, C-11, C-12; H-13/C-8, C-12, C-14, C-17, C-18, C-27; H-19/C-18, C-20, C-21, C-22, C-29, C-30; H-2′/C-1, C-1′, C-3′, C-4′; H-4′/C-2′, C-3′, C-5′, C-6′; H-16′/C-14′, C-15′. This suggested the possible replacement of the hydroxy group at the C-3′ position in **5** with an acetoxy group in **1**. Alkaline hydrolysis of **1** furnished the known compounds lupeol and 3-hydroxyhexadacanoic acid,[13]

Table 2. ^{13}C NMR data for compounds **1**–**4** ($CDCl_3$)[a]

Carbon	1	2	3	4
1	38.5	38.5	38.4	38.4
2	23.8	23.7	23.8	23.7
3	81.5	82.4	81.4	82.3
4	37.9	37.9	37.9	37.9
5	55.5	55.5	55.5	55.4
6	18.3	18.3	18.3	18.3
7	34.3	34.3	34.2	34.2
8	41.0	40.9	40.9	40.8
9	50.4	50.4	50.3	50.3
10	37.2	37.2	37.2	37.2
11	21.0	21.0	21.0	21.0
12	25.2	25.2	25.2	25.2
13	38.2	38.1	37.1	37.2
14	43.0	42.9	42.8	42.8
15	27.6	27.5	27.4	27.4
16	35.7	35.7	35.1	35.0
17	43.1	43.1	43.2	43.3
18	48.4	48.4	49.8	49.8
19	48.1	48.1	52.8	52.8
20	151.0	151.1	213.0	212.9
21	29.8	29.7	29.1	29.1
22	40.1	40.1	40.0	39.9
23	28.0	28.1	28.0	28.0
24	16.6	16.6	16.6	16.6
25	16.1	16.1	16.0	16.0
26	16.2	16.2	16.2	16.2
27	14.6	14.6	14.5	14.5
28	18.1	18.1	18.1	18.1
29	109.5	109.4		
30	19.4	19.3	23.7	23.6
1′	170.3	167.2	170.3	167.1
2′	39.6	49.8	39.7	49.8
3′	70.9	203.2	71.0	203.1
4′	34.0	43.3	34.0	43.3
5′–13′	29.3–29.7	29.2–29.7	29.3–29.8	29.3–29.8
14′	32.0	32.0	32.0	32.0
15′	22.8	22.8	22.8	22.8
16′	14.2	14.2	14.2	14.2
O*CO*CH_3	170.4		170.4	
OCO*CH_3*	21.3		21.3	

[a] Assignments made on the basis of COSY, HMQC, and HMBC and comparison with the literature data.[12,14]

Table 1. ^1H NMR data for compounds **1**–**4** ($CDCl_3$)[a]

H	1	2	3	4
3	4.47 (dd, 10.7, 5.5)	4.52 (dd, 11.3, 4.9)	4.47 (dd, 11.0, 5.5)	4.52 (dd, 11.4, 5.5)
19	2.37 (dt, 5.8, 11.0)	2.35 (m)	2.54 (m)	2.56 (m)
23	0.84 (s)	0.84 (s)	0.84 (s)	0.87 (s)
24	0.82 (s)	0.83 (s)	0.83 (s)	0.83 (s)
25	0.82 (s)	0.83 (s)	0.83 (s)	0.81 (s)
26	1.02 (s)	1.02 (s)	1.01 (s)	1.01 (s)
27	0.93 (s)	0.94 (s)	0.95 (s)	0.95 (s)
28	0.78 (s)	0.78 (s)	0.77 (s)	0.77 (s)
29	4.55 (d, 2.5)	4.56 (d, 2.4)		
	4.67 (d, 2.5)	4.67 (d, 2.4)		
30	1.67 (s)	1.67 (s)	2.14 (s)	2.14 (s)
2′	2.58 (dd, 15.3, 7.7)			
	2.52 (dd, 15.3, 5.3)	3.41 (s)	2.55 (m)	3.41 (s)
3′	5.18 (m)		5.18 (m)	
4′	1.60 (m)	2.51 (t, 7.4)	1.62 (m)	2.51 (t, 7.3)
5′	1.27 (br s)	1.76 (m)	1.28 (br s)	1.78 (m)
6′–15′	1.27 (br s)	1.28 (br s)	1.28 (br s)	1.27 (br s)
16′	0.87 (t, 6.7)	0.87 (t, 6.8)	0.87 (t, 6.7)	0.87 (t, 6.8)
$OCOCH_3$	2.00 (s)		2.01 (s)	

[a] Assignments made on the basis of COSY, HMQC, and HMBC spectral data and in comparison with the literature values.[12,14]

confirming the stereochemistry of the rings in the pentacyclic skeleton of lupeol[6] and the assignment of the acetyl group to the C-3′ position. The presence of the acetyl group was further supported by the HMBC correlations of the acetoxy methyl singlet at δ 2.00 to the carbonyl group at δ 170.5 and the C-3′ carbon resonating at δ 70.9. In addition, the EIMS of **1** contained fragment ions at *m*/*z* 662 $(M-C_2H_4O_2)^+$ and 409 $(M-C_{18}H_{33}O_4)^+$, formed by the loss of acetic acid and 3-acetoxyhexadecanoyl molecules, respectively, from the molecular ion. The absolute stereochemistry of the hydroxy group in 3-hydroxyhexadecanoic acid was assigned as *R* by comparison of its $[\alpha]_D^{25}$ value (−12.3, $CHCl_3$, *c* 1.2) with the value reported in the literature ($[\alpha]_D^{20}$ −12.6, $CHCl_3$, *c* 2.08).[13] On the basis of the above spectral data and chemical conversion studies, the structure of compound **1** was assigned as 3β-(3*R*-acetoxyhexadecanoyloxy)-lup-20(29)-ene.

The molecular formula of **2** was determined as $C_{46}H_{78}O_3$ by HRFABMS. It also gave a positive LB test for triterpenoids. The IR spectrum showed the presence of two carbonyl groups (1735 and 1725 cm^{-1}) similar to **1**. The ^1H NMR spectrum (Table 1) showed the presence of six methyl singlets [δ 0.78 (3H), 0.83 (2 × 3H), 0.84 (3H), 0.94 (3H), 1.02 (3H)], an isopropylene group [4.52 (1H, d, $J = 2.4$ Hz), 4.67 (1H, d, $J = 2.4$ Hz), and 1.67 (s, 3H)], and an oxymethine group [4.56 (1H, dd, $J = 11.3$, 4.9 Hz)] very similar to that in the 3β-substituted lup-20(29)-ene skeleton, the terpenoid part of **1**. The side chain portion showed the presence of a triplet at δ 0.87 (3H, t, $J = 6.8$ Hz), a broad singlet at δ 1.28 for 10 methylene groups, and three more methylene groups centered at δ 1.76 (m), 2.51 (t, $J = 7.4$ Hz), and 3.41 (s). The ^{13}C NMR values for all the carbons in **2** were assigned on the basis of HMQC and HMBC spectral data and are given in Table 2. A comparison of the ^1H and ^{13}C NMR values of **2** with those of **1** indicated the absence of the secondary acetate group in **1**, and the presence of a ketone carbonyl group (δ 203.2), suggesting the possible placement of this group at the C-3′ position in **2**. This was supported by the singlet observed at δ 3.41 for the methylene group flanked between ester and ketone carbonyl groups.[12] The position of the keto group at C-3′ was supported by the key HMBC correlations: H-2′/C-1′, C-3′, C-4′; H-4′/C-2′, C-3′, C-5′, C-6′ and from the mass spectral fragment at *m*/*z* 409 $(M-C_{16}H_{29}O_3)^+$ formed by the loss of a 3-ketohexadecanoyl molecule from the molecular ion. The same compound was previously reported as a synthetic product formed by the Jones oxidation of 3β-(3*R*-hydroxyhexdecanoyloxy)-lup-20(29)ene (**5**),[12] but this is the first report of its occurrence as a natural product; its NMR data (^1H and ^{13}C) have not been reported earlier. Hydrolysis of **2** gave lupeol, identified by co-migration on TLC and by EIMS. Thus, **2** was established as 3β-(3-ketohexadecanoyl)-lup-20(29)-ene.

Compound **3** was isolated as an optically active viscous liquid and was shown to have the molecular formula $C_{47}H_{80}O_5$ from its HRFABMS and ^{13}C NMR spectrum. Its IR spectrum showed the presence of two carbonyl groups similar to those in **1** and **2**. The mass spectral fragments observed at *m*/*z* 664 and 411 in the EIMS formed by the loss of $C_2H_4O_2$ and $C_{18}H_{33}O_4$, respectively, from the molecular ion suggested the presence of a 3-acetoxyhexadecanoic acid side chain in **3**, as in **1**. The ^1H NMR spectrum of **3** was very similar to that of **1** except for the absence of the signals corresponding to the 2-propenyl group at C-19 and the presence of a methyl singlet at δ 2.14, suggesting the presence of an acetyl group at the C-19 position in **3**. This was supported by the presence of signals in its ^{13}C NMR spectrum for a C-20 carbonyl carbon in place of the quaternary C-20 and methylene C-29 sp^2 carbons observed in the spectrum of **1**. A comparison of the ^{13}C NMR values of **3** with those of **1** and of 30-*nor*-lup-3β-ol-20-one[14,15] indicated that **3** was identical to **1** in the side chain moiety, and to 30-*nor*-lup-3β-ol-20-one in the terpenoid moiety. This confirmed the placement of an acetyl group at the C-19 position in **3** in place of the 2-propenyl group in **1**. The ^{13}C NMR values for all the carbons in **3** (Table 2) were assigned on the basis of HMQC and HMBC spectral data and were in good agreement with the structure. Alkaline hydrolysis of **3** furnished 30-*nor*-lup-3β-ol-20-one and 3*R*-hydroxyhexadecanoic acid ($[\alpha]_D^{25}$ −12.0, $CHCl_3$, *c* 0.52). Ozonolysis of **1** furnished a product identical to **3** (TLC, $[\alpha]_D^{25}$ and ^1H NMR), confirming the *R* stereochemistry of the secondary acetate group at the C-3′ position. The above spectral data and chemical studies established the structure of **3** as 3β-(3*R*-acetoxyhexadecanoyloxy)-29-*nor*-lupan-20-one.

The molecular formula of compound **4** was deduced as $C_{45}H_{76}O_4$ by HRFABMS and ^{13}C NMR spectra. The mass spectral fragment observed at *m*/*z* 411 in the EIMS suggested the presence of a 3-ketohexadecanoyl side chain similar to that of **2**. A comparison of the ^1H NMR spectral of **4** with those of **2** and **3** (Table 1) suggested that **4** was identical to **2** in the side chain moiety and to **3** in the terpenoid moiety. The ^{13}C NMR values for all the carbons (Table 2) were assigned in comparison with compounds **2** and **3** and are in good agreement with the structure. Ozonolysis of **2** furnished a product, which was identified as **4** on the basis of their identical R_f values on TLC and their identical ^1H NMR spectra. On the basis of the above data, the structure of **4** was established as 3β-(3-ketohexadecanoyloxy)-29-*nor*-lupan-20-one.

Purified compounds were used to determine IC_{50} values for inhibition of the lyase activity of rat DNA polymerase β. The lyase assay employed a positive control in the form of a crude extract that strongly inhibited deoxyribose phosphate excision. As shown in Table 3, all of the compounds displayed quite reasonable activity, but compounds **1**, **3**, **4**, and lupeol were exceptionally active.

These results suggest that the presence of a lipophilic ester at the 3-position of the lupane skeleton generally seemed to have a facilitating effect on the lyase activity. The exception was compound **2** which had a keto group within this substituent, and was somewhat less active than **1**, **3**, and **4**. Interestingly lupeol, which lacked the

Table 3. IC_{50} of polymerase β lyase inhibition of compounds isolated from *S. canadensis*[a]

Compound	IC_{50} (μM)
1	3.8
2	21.5
3	5.3
4	4.5
Lupeol	6.4
Lupeyl acetate	20.6
Ursolic acid	26.4
Cycloartenol	22.6
Cycloartenyl palmitate	21.8
α-Amyrin acetate	24.4
Stigmasterol	26.8

[a] Data are the mean of three determinations.

lipophilic ester substituent, also displayed strong inhibitory activity.

3. Experimental section

3.1. General experimental methods

Melting points were recorded with an electrothermal digital apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR (KBr) and UV (MeOH) spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on a JEOL Eclipse 500 spectrometer. The HRFABMS were obtained on a JEOL HX-110 instrument. The chemical shifts are given in ppm (δ) with TMS (tetramethylsilane) as an internal reference, and coupling constants (J) are in Hz.

3.2. Bioassay studies

The polymerase-β lyase assay was performed as previously reported.[16]

3.3. Plant material

Roots and stems of *Solidago canadensis* L. (Asteraceae) were obtained from the National Cancer Institute.

3.4. Extract preparation

The plant samples were dried, ground, soaked with MEK and evaporated to give the dried MEK extract PC-6-93.

3.5. Extraction and isolation

The crude MEK extract (0.65 g) was suspended in aqueous MeOH (9:1 MeOH–H_2O, 100 mL) and extracted with three portions of 50 mL hexane. The aqueous layer was then diluted to 70% MeOH (v/v) with H_2O and extracted with three portions of 50 mL $CHCl_3$. The aqueous layer was concentrated and the residue obtained was suspended in H_2O (25 mL) and extracted with two 25-mL portions of *n*-BuOH. The hexane and $CHCl_3$ extracts were found to be equally active and were combined on the basis of their similar nature on TLC and their similar ^1H NMR patterns. The combined residue (0.53 g) was fractionated over MCI gel using MeOH/H_2O (1:1 to 100:0) to furnish ten fractions (A–J), of which fractions A–E and G–H were further fractionated on the basis of their activity and ^1H NMR spectra. Fraction A on reversed-phase preparative TLC (MeOH–H_2O, 80:20) yielded cycloartenyl palmitate (15.0 mg). Fraction B on reversed-phase preparative TLC (MeOH–H_2O, 80:20) yielded the new triterpene **1** (16.4 mg). Similarly, fraction C on reversed-phase preparative TLC (MeOH–H_2O, 85:15) yielded the two known compounds α-amyrin acetate (12.2 mg) and stigmasterol (10.4 mg), in addition to two active fractions C-3 and C-4, which on normal phase HPLC with the mobile phase $CHCl_3$–MeOH (100:1) furnished the two new triterpenes **3** (3.4 mg) and **4** (1.5 mg). Fractions D and E were combined on the basis of their identical TLC pattern and the combined fraction on reversed-phase preparative TLC (MeOH–H_2O, 90:10) yielded the two known compounds ursolic acid (6.2 mg) and lupeol (8.8 mg). Fraction G on reversed-phase preparative TLC (MeOH–H_2O, 85:15) yielded the two known compounds cycloartenol (4.6 mg) and lupeyl acetate (3.6 mg). Fraction H on reversed-phase preparative TLC (MeOH–H_2O, 98:2), followed by normal phase HPLC with the mobile phase $CHCl_3$–MeOH (200:1), furnished the new triterpene **2** (1.8 mg). The structures of the known compounds were identified by comparison of their spectral data with the literature values.[6–10]

3.6. 3β-(3*R*-Acetoxyhexadecanoyloxy)-lup-20(29)-ene (1)

Colorless solid; $[\alpha]_D^{25}$ +42.2 (c 0.06, $CHCl_3$); UV (MeOH) λ_{max} ($\log\varepsilon$) 215 (3.24) nm; IR ($CHCl_3$) ν_{max} 2945, 1732, 1723 1450, 1145, 1100, 1050, 850 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR see Table 2; EIMS *m/z*: 722 $[M]^+$ (8), 662 (12), 409 (14), 352 (76), 349 (18), 205 (12), 190 (8), 171 (23), 93 (100); HRFABMS *m/z* 723.6291 $[M+H]^+$ (calcd for $C_{48}H_{83}O_4$ 723.6294).

3.7. Alkaline hydrolysis of 3β-(3*R*-acetoxyhexadecanoyloxy)-lup-20(29)-ene (1)

To a solution of compound **1** (3.5 mg) in $CHCl_3$ (5 mL) was added 5 mL of 1.2 N NaOH and the reaction mixture was heated at reflux for 6 h. The reaction mixture was allowed to cool to room temperature and the organic layer was separated. The aqueous layer was extracted two more times with 10 mL portions of $CHCl_3$. The combined organic layer, which was concentrated and purified by preparative TLC ($CHCl_3$), furnished a colorless solid that was identified as lupeol (1.8 mg) by spectral data (^1H and ^{13}C NMR).[6] The aqueous layer was acidified to pH 2.0 with 1 N HCl and extracted with three 10 mL portions of diethyl ether to yield 3*R*-hydroxyhexadacanoic acid (0.8 mg), which was identified by its ^1H NMR and $[\alpha]_D^{25}$ values.[13]

3.8. Ozonolysis of 3β-(3*R*-acetoxyhexadecanoyloxy)-lup-20(29)-ene (1)

A stream of ozone was passed into a solution of **1** (3.5 mg) in dry CH_2Cl_2 (3 mL) at −78 °C for 15 min.

Then the solution was saturated with nitrogen gas for 5 min, and the mixture was allowed to warm to −25 °C. The reaction mixture was treated with triphenylphosphine (5 mg) and stirred at −25 °C for 10 h. The reaction mixture was allowed to warm to room temperature, stirred for 1 h and concentrated under vacuum. The residue obtained was purified by preparative TLC (50:1 $CHCl_3$–MeOH) to furnish a product, which was identified as **3** (1.6 mg) by co-TLC, $[\alpha]_D^{25}$, and ^{1}H NMR spectral data.

3.9. 3β-(3-Ketohexadecanoyloxy)-lup-20(29)-ene (2)

Viscous oil; $[\alpha]_D^{25}$ +18.8 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 223 (3.24) nm; IR ($CHCl_3$) ν_{max} 2955, 1735, 1725 1460, 1130, 1080, 1040, 840 cm^{-1}; ^{1}H NMR, see Table 1; and ^{13}C NMR see Table 2; EIMS *m*/*z*: 678 [M]$^+$ (21), 409 (28), 352 (54), 349 (19), 205 (21), 190 (13), 171 (28), 93 (100); HRFABMS *m*/*z* 679.6029 [M+H]$^+$ (calcd for $C_{46}H_{79}O_3$ 679.6032).

3.10. Ozonolysis of 3β-(3-ketohexadecanoyloxy)-lup-20(29)-ene (2)

Ozonolysis of **2** (0.8 mg) as mentioned above, followed by purification of the residue obtained by preparative TLC ($CHCl_3$–MeOH, 100:1), furnished a product, which was identified as **4** (0.4 mg) by co-TLC and ^{1}H NMR spectral data.

3.11. 3β-(3*R*-Acetoxyhexadecanoyloxy)-29-*nor*-lupan-20-one (3)

Viscous oil; $[\alpha]_D^{25}$ +24.5 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 216 (4.21) nm; IR ($CHCl_3$) ν_{max} 2945, 1735, 1723, 1120, 1085, 1035, 940, 860, 760 cm^{-1}; ^{1}H NMR, see Table 1; and ^{13}C NMR see Table 2; EIMS *m*/*z*: 724 [M]$^+$ (12), 664 (12), 411 (32), 374 (76), 217 (14), 205 (16), 191 (12), 171 (26), 93 (100); HRFABMS *m*/*z* 724.6006 [M]$^+$ (calcd for $C_{47}H_{80}O_5$ 724.6009).

3.12. Alkaline hydrolysis of 3β-(3*R*-acetoxyhexadecanoyloxy)-29-*nor*-lupan-20-one (3)

Hydrolysis of **3** (1.6 mg) as reported above furnished 29-*nor*-lup-3β-ol-20-one (0.6 mg)[14] and 3*R*-hydroxyhexadecanoic acid (0.3 mg).[13]

3.13. 3β-(3-Ketohexadecanoyloxy)-29-*nor*-lupan-20-one (4)

Viscous oil; $[\alpha]_D^{25}$ +12.5 (*c* 0.21, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 225 (3.46) nm; IR ($CHCl_3$) ν_{max} 2925, 1738, 1722, 1465, 1135, 1105, 1065, 1050, 1020, 935, 750 cm^{-1}; ^{1}H NMR, see Table 1; and ^{13}C NMR see Table 2; EIMS *m*/*z*: 680 [M]$^+$ (11), 411 (42), 382 (12), 369 (16), 204 (9), 191 (12), 171 (27), 93 (100); HRFABMS *m*/*z* 680.5744 [M]$^+$ (calcd for $C_{45}H_{76}O_4$ 680.5744).

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