OXIDATIVE CLEAVAGE OF 3β-ACETOXY-21-OXOLUP-18-EN-28-OIC ACID BY RUTHENIUM TETROXIDE

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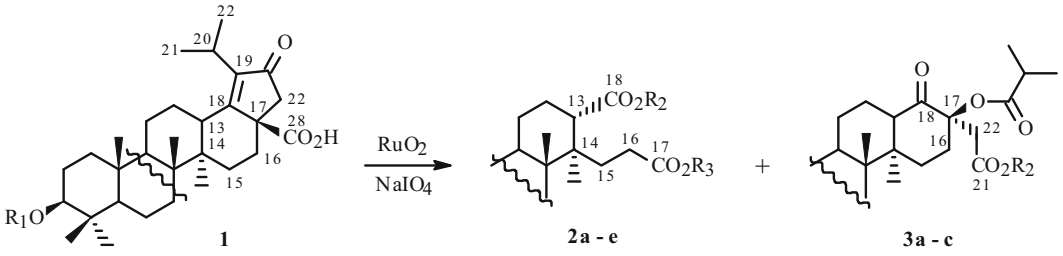
*3*β*-Acetoxy-21-oxolup-18-en-28-oic acid was used as a starting compound for oxidative cleavage by ruthenium tetroxide generated* in situ *from ruthenium dioxide by sodium metaperiodate in a biphasic system. Tricyclic and tetracyclic acids of baccharane type were prepared by this reaction, and their structures were confirmed using spectral data. These acids were further used for synthesis of appropriate methyl and acetoxymethyl esters. All prepared compounds were tested on cytotoxic activity against CEM tumor cell line, and several compounds showed activity in low micromolar concentrations.*

Keywords: lupane triterpenoids, oxidation, ruthenium tetroxide, cleavage, decarboxylation, cytotoxicity.

Natural pentacyclic triterpenes of the lupane series are studied as compounds with interesting biological activities [1]. Previously we have reported many lupane derivatives with significant cytotoxic activity [2–4]. Oxidative cleavage of the 18,19-double bond with ruthenium tetroxide was found to be the appropriate way to synthesize new baccharane or des-*E*-lupane derivatives [2–5]. Within our research, we have applied the oxidative cleavage on 3β-acetoxy-21-oxolup-18-en-28-oic acid (**1**) which is easily accessible by a five-step synthesis from betulin [2].

Prolonged treatment of acid **1** with ruthenium tetroxide, prepared *in situ* from ruthenium dioxide and $NaIO_4$ in a biphasic system of ethyl acetate and water, afforded two major compounds, heptanordiacid **2a** and 28-noracid **3a** (Scheme 1). While the stereochemistry of heptanordiacid **2a** is apparent due to the skeletal configuration, in the case of 28-noracid **3a** the configuration of C-17 had to be resolved with 2D NMR spectra, especially ROESY: cross-peaks among hydrogens of the isobutyryl group and H-13β were proof of the 17*S*-configuration.



1: R_1 = Ac; **2a:** R_1 = Ac, R_2 = R_3 = H; **2b:** R_1 = Ac, R_2 = R_3 = CH_3
2c: R_1 = R_3 = H, R_2 = CH_3; **2d:** R_1 = Ac, R_2 = CH_3, R_3 = H
2e: R_1 = Ac, R_2 = CH_3, R_3 = CH_2OAc

3a: R_1 = Ac, R_2 = H
3b: R_1 = Ac, R_2 = CH_3
3c: R_1 = Ac, R_2 = CH_2OAc

Scheme 1

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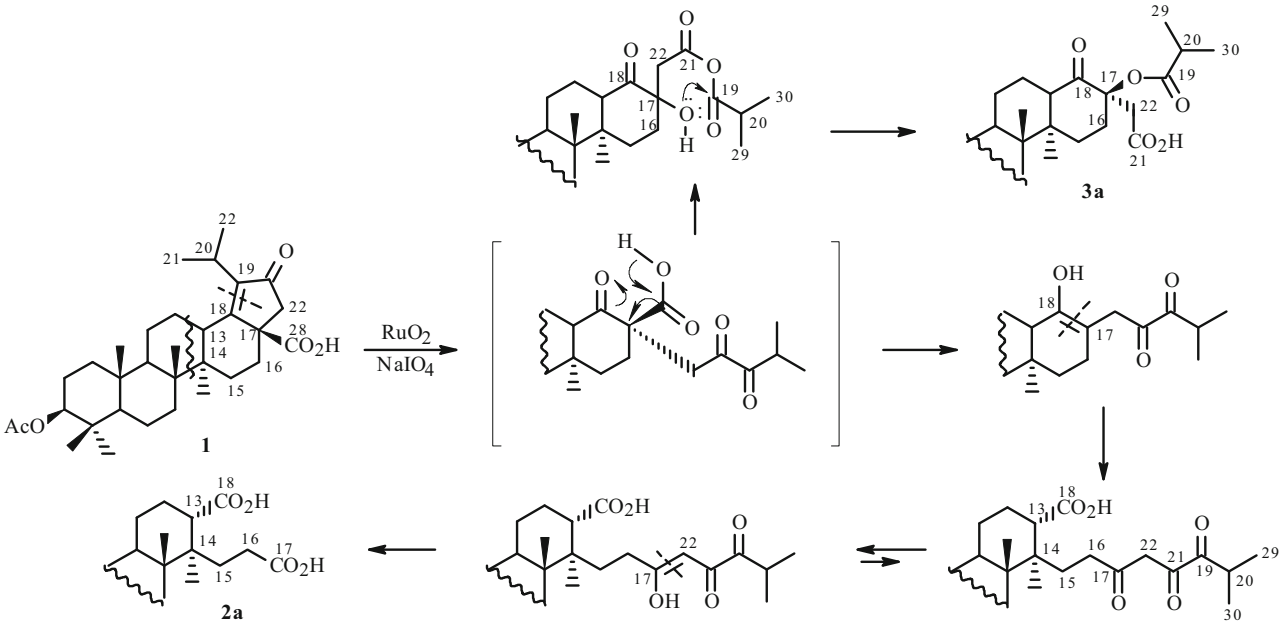

Scheme 2

According to the proposed mechanism, ketoacid **1** undergoes a slow oxidative cleavage that leads to two final products – heptanoracid **2a** and 28-noracid **3a**. Formation of both acid **2a** and **3a** could be explained in Scheme 2. Apparently there is a cleavage of the 18(19) double bond of 21-oxoacid **1** with ruthenium (VIII) oxide, which produces the 18,19,21-trioxoacid. This acid then reacts further in two ways. In the first way, after decarboxylation to 28-notriketone, there is a cleavage of the enolic 17(18)-double bond, affording the 17,19,21-trioxoacid with an easily enolized β-diketone system. The last step is cleavage of the enolic 17(22)-double bond, leading to heptanoracid **2a**. In the second way, oxidative decarboxylation at C-17 may occur. This is then followed by oxidative cleavage of the 19,21-diketone to a mixed anhydride. The free hydroxyl group at C-17 then undergoes an intramolecular esterification to afford 28-noracid **3a**.

According to our previous results [6] we also tried to prepare acetoxymethyl (Acm) esters for cytotoxicity screening. Acm-esters were previously shown to better penetrate cellular membranes than free acids and to release free compounds after intracellular enzymatic deprotection, as demonstrated on penicillins [7]. Methyl esters (**2b**, **3b**) and free acids (**2a**, **3a**) were used as standards for comparison of the biological activity.

The attempt to prepare the bis-Acm ester of diacid **2a** has failed due to the low stability of this compound. Hence, we tried to prepare at least the mono-Acm ester. The mild basic hydrolysis of dimethyl ester **2b** afforded only the expected monoester **2c**; on acetylation, acetate **2d** was formed. Compound **2d** was a suitable precursor for the preparation of Acm ester **2e**. Synthesis of Acm ester **3c** could be done within one step.

Cytotoxicity Activity. All the above products were tested for cytotoxic activity on the *T*-lymphoblastic leukemia cell line (CEM) using the MTT assay [8, 9], while normal human fibroblasts BJ were used to evaluate the activity of the compounds on non-malignant cells. The lowest cytotoxic activity was found in the case of compounds **2c** (137 μmol/L) and **2d** (193 μmol/L), whereas heptanor derivatives **2a**, **2b** and **2e** were inactive (>250 μmol/L) against the CEM cell line. On the other hand, acid **3a** was found to be the most active compound prepared in this work (6.8 μmol/L on CEM cells). Compound **3a** is a perspective candidate for broad screening against other tumor cell lines. However, other 18-oxo derivatives (**3b**, **3c**) showed no significant cytotoxic activity against the leukemia cell line. Interestingly, triterpenic derivatives showed impressive selectivity against CEM leukemia cells, since they were not cytotoxic against normal human fibroblasts BJ.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotatory power was measured on an Autopol III (Rudolph Research, Flanders, NJ) polarimeter as $CHCl_3$ solutions (if not otherwise stated). ^1H and ^{13}C NMR spectra were recorded on Varian ^{UNITY} Inova 400 (400 MHz, for ^1H), using $CDCl_3$ as a solvent (if not otherwise stated). Chemical shifts are expressed in ppm with tetramethylsilane as an internal standard for ^1H spectra. ^{13}C NMR spectra are

referenced to $CDCl_3$ (77.00 ppm). Mass spectra (EI) were measured on an INCOS 50 (Finnigan MAT) mass spectrometer. IR spectra were recorded on a Nicolet Avatar 370 FT-IR spectrometer, using $CHCl_3$ as a solvent (if not otherwise stated). TLC was performed on Kieselgel 60 F_{254} (Merck) sheets, detected by spraying 10% sulfuric acid with heating to 110–200°C. Elemental analyses of all compounds corresponded to those calculated.

Oxidation of Acid 1 with RuO_4. A solution of acid **1** (1.32 mmol, 0.6 g) in ethyl acetate (30 mL) and CH_3CN (10 mL) was added to a mixture of RuO_2 (0.33 mmol, 45 mg), $NaIO_4$ (25.2 mmol, 4.2 g), H_2O (30 mL), and trifluoracetic acid (1 mL). The two-phase mixture was stirred vigorously at room temperature for 4 days. The reaction was monitored with TLC ($CHCl_3$–EtOAc–AcOH 100:20:1). After the reaction was complete, isopropyl alcohol was added. The mixture was filtered, and the phases were separated. The organic layer was diluted with ethyl acetate, washed with a solution of sodium thiosulfate and water, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. Separation of products by preparative TLC ($CHCl_3$–EtOAc–AcOH 100:20:1) gave heptanordiacid **2a** and 28-noracid **3a**.

3β-Acetoxy-17,18-*seco*-19,20,21,22,28,29,30-heptanorbaccharan-17,18-dioic acid (2a): yield 252 mg (50%), mp 252–254°C, $[\alpha]_D^{20}$ –1.0° (*c* 0.56; dioxane); lit. [5] records mp 246–248°C (hexane–acetone), $[\alpha]_D^{20}$ –4.7 (*c* 0.42; $CHCl_3$). $C_{25}H_{40}O_6$.

3β-Acetoxy-17β-isobutyryloxy-18-oxo-19,20,28,29,30-pentanorbaccharan-21-oic acid (3a): yield 159 mg (26%), mp 109–113°C (diethyl ether), $[\alpha]_D^{20}$ +37.0° (*c* 0.37; $CHCl_3$). $C_{31}H_{48}O_7$. IR spectrum (ν, cm^{-1}): 3620, 2400–3300, 1731, 1255.

PMR spectrum (δ, ppm, J/Hz): 0.83, 0.84, 0.85, 0.88, 1.10 (s, $5CH_3$), 1.19 and 1.23 (6H, both d, J = 7.0, $2CH_3$), 2.04 (3H, s, OAc), 1.83 (1H, ddd, $J_{16\alpha16\beta}$ = 14.7, $J_{16\alpha15\beta}$ = 13.6, $J_{16\alpha15\alpha}$ = 4.3), 2.10 (1H, dt, $J_{15\beta15\alpha}$ = 13.3, $J_{15\beta16\alpha}$ = 13.3, $J_{15\beta16\beta}$ = 4.0), 2.46 (1H, ddd, $J_{16\beta16\alpha}$ = 14.7, $J_{16\beta15\beta}$ = 4.0, $J_{16\beta15\alpha}$ = 2.6), 2.61 (1H, septet, J = 7.0, H-32), 2.75 (1H, m, H-13β), 2.80 and 3.05 (2H, both d, J = 14.5, H-22).

Mass spectrum (EI, 70 eV), *m*/*z* (I_{rel}, %): 532 $[M]^+$ (1), 472 (14), 457 (7), 444 (18), 384 (25), 341 (22), 249 (21), 203 (26), 189 (100).

Dimethyl 3β-Acetoxy-19,20,21,22,28,29,30-heptanor-17,18-*seco*-baccharan-17,18-dioate (2b). A solution of diazomethane (0.2 mol/L) in diethyl ether (10 mL) was added to a solution of heptanordiacid **2a** (0.44 mmol, 200 mg) in chloroform (5 mL). After 30 min, the solvents were evaporated and the product crystallized from MeOH. Yield 200 mg (95%), mp 143–145°C, $[\alpha]_D^{20}$ +16° (*c* 0.15; $CHCl_3$). Lit. [5] mp 140–144°C (MeOH), $[\alpha]_D^{20}$ +12.2° (*c* 1.0; $CHCl_3$). $C_{27}H_{44}O_6$.

Methyl 3β-acetoxy-17β-isobutyryloxy-18-oxo-19,20,28,29,30-pentanorbaccharan-21-oate (3b) was prepared analogously to **2b** from **3a** (0.09 mmol, 50 mg). Yield 47 mg (94%), mp 202–204°C (MeOH), $[\alpha]_D^{20}$ +39.0° (*c* 0.17; $CHCl_3$). $C_{32}H_{50}O_7$. IR spectrum (ν, cm^{-1}): 1731, 1255.

PMR spectrum (δ, ppm, J/Hz): 0.83, 0.84, 0.85, 0.88, 1.09 (s, $5CH_3$), 1.20 and 1.23 (6H, both d, J = 7.0, $2CH_3$), 1.85 (1H, ddd, $J_{16\alpha16\beta}$ = 14.7, $J_{16\alpha15\beta}$ = 13.5, $J_{16\alpha15\alpha}$ = 4.3), 2.04 (3H, s, OAc), 2.08 (1H, dt, $J_{15\beta15\alpha}$ = 13.2, $J_{15\beta16\alpha}$ = 13.2, $J_{15\beta16\beta}$ = 4.0), 2.43 (1H, ddd, $J_{16\beta16\alpha}$ = 14.7, $J_{16\beta15\beta}$ = 4.0, $J_{16\beta15\alpha}$ = 2.4), 2.60 (1H, septet, J = 7.0, H-32), 2.74 (1H, m, H-13β), 2.70 and 3.08 (2H, both d, J = 14.7, H-22), 3.66 (3H, s, OCH_3).

Mass spectrum (EI, 70 eV), *m*/*z* (I_{rel}, %): 546 $[M]^+$ (2), 471 (2), 458 (42), 443 (7), 383 (14), 345 (5), 251 (9), 229 (16), 189 (100).

3β-Hydroxy-13α-methoxycarbonyl-18,19,20,21,22,28,29,30-octanor-17,18-*seco*-baccharan-17-oic Acid (2c). KOH (1.25 mmol, 50 mg) was added to a solution of diester **2b** (0.32 mmol, 150 mg) in MeOH (5 mL) and water (0.5 mL) and the mixture was refluxed for 1 h. Then it was acidified by 10% aqueous HCl and extracted with ethyl acetate, and the collected organic layers were washed twice with water and dried over $MgSO_4$. The solvent was evaporated and the crude product was crystallized from MeOH. Yield 121 mg (92%), mp 178–182°C (MeOH), $[\alpha]_D^{20}$ +13° (*c* 0.10; DMF). $C_{24}H_{40}O_5$. IR spectrum in KBr (ν, cm^{-1}): 3563, 1730, 1718.

PMR spectrum in CD_3OD (δ, ppm, J/Hz): 0.77, 0.89, 0.96, 1.04, 1.06 (s, $5CH_3$), 1.87 (1H, td, J_{15a15b} = 11.6, J_{15a16a} = 11.6, J_{15a16b} = 5.7), 1.93 (1H, td, J_{16a16b} = 11.6, J_{16a15a} = 11.6, J_{16a15b} = 4.0), 2.12 (1H, m, H-15b), 2.67 (1H, dd, $J_{13\beta12\alpha}$ = 12.8, $J_{13\beta12\beta}$ = 3.7), 3.15 (1H, dd, $J_{3\alpha2\beta}$ = 11.2, $J_{3\alpha2\alpha}$ = 5.6), 3.62 (3H, s, OCH_3).

Mass spectrum (EI, 70 eV), *m*/*z* (I_{rel}, %): 408 $[M]^+$ (1), 390 (3), 390 (86), 375 (45), 331 (39), 207 (76), 189 (100).

3β-Acetoxy-13α-methoxycarbonyl-18,19,20,21,22,28,29,30-octanor-17,18-*seco*-baccharan-17-oic Acid (2d). Acetic anhydride (2 mL) was added to a solution of acid **2c** (0.25 mmol, 100 mg) in pyridine (2 mL). The reaction mixture was held at room temperature for 12 h and then diluted with ethyl acetate, extracted twice with 10% aqueous HCl, washed twice with water, and dried over $MgSO_4$. The solvent was evaporated and the crude product was crystallized from MeOH. Yield 103 mg (94%), mp 172–174°C (MeOH), $[\alpha]_D^{20}$ +8° (*c* 0.16; $CHCl_3$). $C_{26}H_{42}O_6$. IR spectrum (ν, cm^{-1}): 3517, 1723, 1715, 1256.

PMR spectrum (δ, ppm, J/Hz): 0.84, 0.86, 0.87, 1.00, 1.05 (s, 5CH_3), 1.88 (1H, td, J = 14.0, J′ = 6.0), 2.02 (1H, td, J_{16a16b} = 15.6, J_{16a15a} = 15.6, J_{16a15b} = 4.4), 2.05 (3H, s, OAc), 2.21 (1H, ddd, J_{16b16a} = 15.6, J_{16b15a} = 10.7, J_{16b15b} = 5.5), 2.63 (1H, dd, $J_{13\beta12\alpha}$ = 12.4, $J_{13\beta12\beta}$ = 3.9), 3.64 (3H, s, OCH_3), 4.48 (1H, dd, $J_{3\alpha2\beta}$ = 11.2, $J_{3\alpha2\alpha}$ = 5.3).

Mass spectrum (EI, 70 eV), *m*/*z* (I_{rel}, %): 450 $[M]^+$ (1), 420 (3), 390 (54), 375 (23), 347 (31), 331 (28), 189 (100).

Acetoxymethyl 3β-Acetoxy-13α-methoxycarbonyl-18,19,20,21,22,28,29,30-octanor-17,18-*seco*-baccharan-17-oate (2e). AcmBr (0.14 mmol, 22 μL) was added to a solution of DBU (0.14 mmol, 22 μL) and acid **2d** (0.13 mmol, 60 mg) in 3 mL CH_2Cl_2 and 1 mL MeCN. The reaction mixture was worked up after 10–12 h. The crude product was then purified by HPLC (hexane–EtOAc 17:3) and then crystallized from MeOH. Yield 56 mg (83%), mp 142–143°C (MeOH), $[\alpha]_D^{20}$ +16° (*c* 0.32; $CHCl_3$). $C_{29}H_{46}O_8$. IR spectrum (ν, cm^{-1}): 1762, 1725, 1255, 1026.

PMR spectrum (δ, ppm, J/Hz): 0.84, 0.85, 0.87, 0.99, 1.03 (s, 5CH_3), 1.89 (1H, m, H-15a), 2.01 (1H, td, J_{16a16b} = 15.4, J_{16a15a} = 15.4, J_{16a15b} = 4.0), 2.04 and 2.11 (6H, both s, 2OAc), 2.23 (1H, ddd, J_{16b16a} = 15.4, J_{16b15a} = 10.1, J_{16b15b} = 5.5), 2.62 (1H, dd, $J_{13\beta12\alpha}$ = 12.3, $J_{13\beta12\beta}$ = 4.5), 3.63 (3H, s, OCH_3), 4.48 (1H, dd, $J_{3\alpha2\beta}$ = 10.8, $J_{3\alpha2\alpha}$ = 5.1), 5.72 (2H, s, OCH_2O).

Mass spectrum (EI, 70 eV), *m*/*z* (I_{rel}, %): 522 $[M]^+$ (1), 462 (69), 447 (23), 419 (30), 389 (27), 345 (28), 331 (28), 189 (100).

Acetoxymethyl 3β-acetoxy-17β-isobutyryloxy-18-*oxo*-19,20,28,29,30-pentanorbaccharan-21-oate (3c) was prepared analogously to **2c** from **3a** (0.09 mmol, 50 mg). Yield 44 mg (78%), mp 132–135°C (MeOH), $[\alpha]_D^{20}$ +41.0° (*c* 0.27; $CHCl_3$). $C_{34}H_{52}O_9$. IR spectrum (ν, cm^{-1}): 1764, 1734, 1721, 1254.

PMR spectrum (δ, ppm, J/Hz): 0.83, 0.84, 0.85, 0.88, 1.09 (s, 5CH_3), 1.20 and 1.24 (6H, both d, J = 7.0, 2CH_3), 2.04 and 2.11 (6H, both s, OAc), 1.88 (1H, ddd, $J_{16\alpha16\beta}$ = 14.6, $J_{16\alpha15\beta}$ = 13.6, $J_{16\alpha15\alpha}$ = 4.2), 2.08 (1H, dt, $J_{15\beta15\alpha}$ = 13.0, $J_{15\beta16\alpha}$ = 13.0, $J_{15\beta16\beta}$ = 4.0), 2.40 (1H, ddd, $J_{16\beta16\alpha}$ = 14.7, $J_{16\beta15\beta}$ = 4.1, $J_{16\beta15\alpha}$ = 2.6), 2.60 (1H, septet, J = 7.0, H-32), 2.73 (1H, m, H-13β), 2.80 and 3.08 (2H, both d, J = 15.0, 2H-22), 5.72 and 5.73 (2H, both d, J = 5.7, OCH_2O).

Mass spectrum (EI, 70 eV), *m*/*z* (I_{rel}, %): 604 $[M]^+$ (not found), 529 (4), 516 (32), 456 (39), 445 (14), 384 (10), 341 (16), 251 (32), 229 (16), 189 (100).

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