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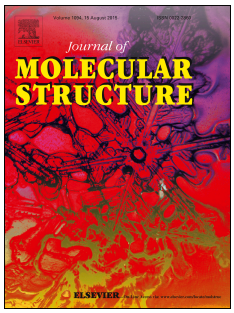

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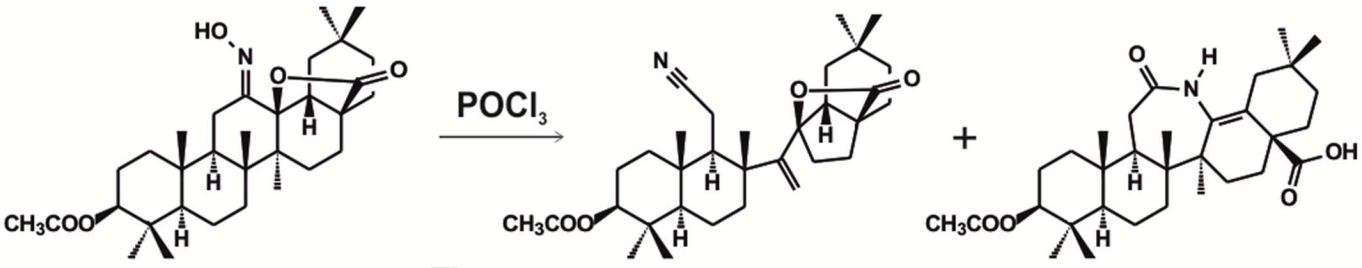

Beckmann rearrangement within the ring *C* of oleanolic acid lactone: synthesis, structural study and reaction mechanism analysis

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Highlights

- 3β-Acetoxy-12-hydroxyimino-18β-oleanan-28,13β-olide was synthesized and used as a substrate in Beckmann rearrangement reaction within ring *C* of oleanane skeleton.
- IR, ^{1}H- and ^{13}C-NMR spectroscopy and X-ray analysis for Beckmann rearrangement substrate (oxime) and products (nitrile and lactam) were performed.
- In nitrile unexpected tandem rearrangements within rings *C* and *D* leading to the cleavage of the first one and the contraction of the latter were observed.
- In obtained lactam the hydrolysis of lactone ring and formation of double bond in *D* ring were observed.

Keywords

Oleanane triterpenoids; ^{1}H- and ^{13}C-NMR, IR spectroscopy, X-ray analysis; Beckmann rearrangement; triterpene lactam; triterpene nitrile; 1,2-rearrangement; triterpenoid ring *D* contraction

Abstract

Synthesis, spectral and X-ray analysis of three compounds, *i.e.* 3β-acetoxy-12-hydroxyimino-18β-oleanan-28,13β-olide (substrate) and 3β-acetoxy-12-nitrile-12,13-seco-15(14→13)-abeoolean-14(27)-en-28,13β-olide and 3β-acetoxy-12-oxo-12a-aza-*C*-homoolean-13(18)-en-28-oic acid (Beckmann rearrangement reaction products) are described. Structural analysis revealed that the oxime group in the ring *C* in substrate molecule had an *E*-configuration. The nitrile product with retained lactone group was a result of major transformations within rings *C* and *D* of oleanane skeleton. In lactam product free carboxyl group and a double bond in ring *D* instead of lactone system were formed in Beckmann rearrangement reaction.

1. Introduction

Oleanolic acid and its derivatives display various interesting biological activities [1-3]. Among many different pharmacological features, hepatoprotective and anti-inflammatory effects of unmodified oleanolic acid and some of its derivatives seem to be the most important ones [4]. It is noteworthy that investigations concerning bardoxolone methyl ester, which was considered as a very promising antioxidative and anti-inflammatory agent for chronic kidney disease in diabetes patients treatment, reached the third stage clinical trials, even though it ended in failure [5]. However, oleanolic acid derivatives show also many other biological properties, *i.e.* anti-proliferative [6], antiviral [7] and antidiabetic [8], to name just a few. In several studies [9-11] this class of compounds was reported to interact with biological membranes, changing their permeability.

This paper is a part of ongoing research aiming at the introduction of lactam system in pentacyclic triterpenoids of oleanane group in order to obtain a novel class of compounds with interesting biological properties. It is commonly known that the activity of N-dodecylcaprolactam (Azone®), one of the most popular and effective transepidermal transport promoters, is associated with the presence of seven-membered lactam ring in its molecule [12]. Therefore, introducing it in the oleanane triterpenoids gives the possibility to enhance their ability to interact with biological membranes. The results of *in vitro* experiments performed for azacyclic oleanane derivatives are described by Zaprutko *et al.* [13]. Some of the analyzed compounds showed activity exceeding the activity of Azone® used as a reference absorption enhancer.

In this paper we present the synthesis and spectral and X-ray analysis of the structures of 3β-acetoxy-12-hydroxyimino-18β-oleanan-28,13β-olide (**5**) as the substrate and nitrile (**6**) and lactam (**7**) as products of Beckmann rearrangement reaction within the ring *C* of oleanane skeleton.

2. Results and discussion

2.1. Synthesis of compounds 2 – 4

The starting material in our experiments, oleanolic acid (**1**), was isolated from by-product residue obtained during the industrial production of mistletoe (*Viscum album*) extract [14]. The purity of the obtained triterpene was proved on the basis of spectral data which were in accordance with the literature spectral characteristics [15]. At this point it is worth noting that the spatial structure of oleanolic acid was solved by Froelich and Gzella [16]. The transformations of the mentioned triterpene are presented in Scheme 1.

Within 18β-oleanan-28,13β-olid derivative with hydroxyl group in C-12 position (Scheme 1, comp. **3**) can be obtained with the use of ozone or $KMnO_4$ [17,18].

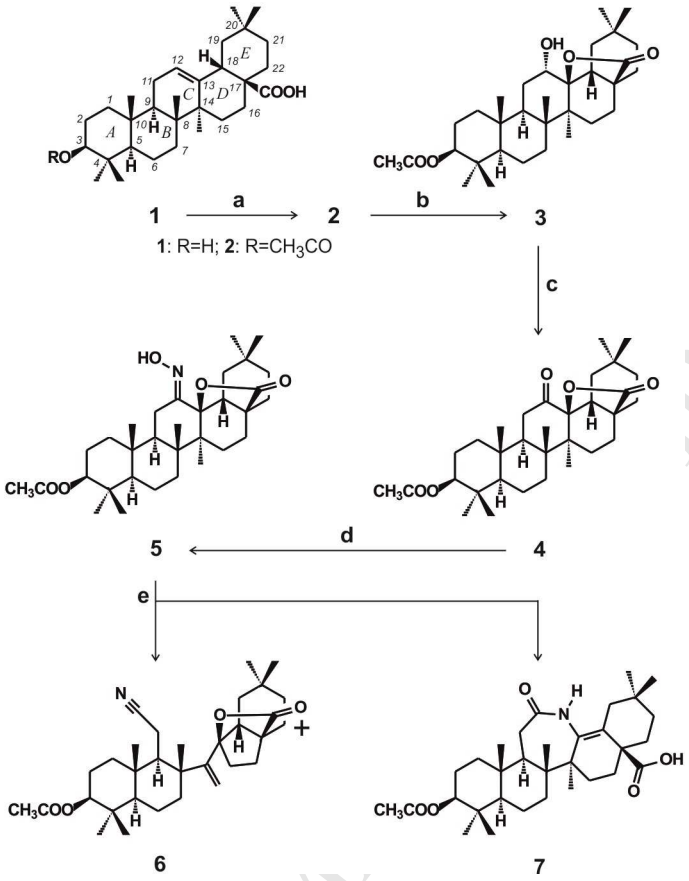
Oxidation of 3-acetoxyoleanolic acid (**2**) with the mixture of $KMnO_4$ and $CuSO_4$ [17] or ozone [18] requires the application of complicated and time-consuming chemical procedures or ozone generator. Moreover, as a result a mixture of products is obtained. As we present in this paper, similar transformation can also be performed with the application of *m*-CPBA in chloroform. By-products formed in this reaction weres removed by the application of extraction with ethyl acetate that allowed us to avoid time-consuming column chromatography.

In the first step of the synthetic route C-3 hydroxyl group of oleanolic acid (**1**) was acylated to acetyloleanolic acid (**2**) with 10-fold excess of acetic anhydride [19]. After that the latter was subjected to the action of *m*-CPBA in chloroform to give 12-hydroxylactone (**3**). The secondary hydroxyl group in the obtained 12-hydroxy-28,13β-lactone (**3**) was oxidized with Jones reagent with application of our method [20] to give the corresponding 12-ketone (**4**). The results of spectral studies of both compounds were in accordance with the literature data [21].

2.2. Synthesis and spectral characterization of compounds 5 – 7

The resulting ketone (**4**) was subjected to the action of hydroxylamine hydrochloride in ethanol to form oxime (**5**). In the last step of the chemical transformations **5** was subjected

to Beckmann rearrangement with $POCl_3$ in dried pyridine to give two products (**6** and **7**) showing different polarity which was proved with TLC analysis.



Scheme 1. Chemical transformations of oleanolic acid leading to oxime (**5**), nitrile (**6**) and lactam (**7**). Reactions and conditions: (a) $(CH_3CO)_2O$, pyridine, rt; 97.5%; (b) *m*-CPBA, $CHCl_3$, 60.6%; (c) Jones reagent, acetone, rt.; 90.6%; (d) $NH_2OH \cdot HCl$, C_2H_5OH, reflux; 95.4%; (e) $POCl_3$, pyridine, rt; 75.7% (for **6**); 20.4% (for **7**).

The structures of **5** - **7** were analyzed with IR, ^{1}H- and ^{13}C-NMR spectroscopy and X-ray crystallography.

In **5** the results of spectral analyses confirmed the presence of 28,13β-lactone system, oxime group in position 12 of ring *C* as well as acetoxy group in C-3 position in ring *A*. The signals observed in ^{13}C-NMR spectrum at 179.45 and 90.76 ppm for C-28 and C-13 atoms indicated the presence of lactone system, while the signals at 171.03 and 80.64 ppm were assigned to C-31 and C-3 atoms of acetoxy group. In IR spectrum two absorption bands at carbonyl region at 1770 and 1730 cm^{-1} were observed. The first can be attributed to the

lactone system and the other one to the acetoxy function [22]. As far as oxime group is concerned, in ^{1}H-NMR spectrum singlet signal with intensity of 1H at 7.60 ppm was found. It was assigned to the hydrogen atom of OH group. In ^{13}C-NMR spectrum a signal from C-12 atom was found at 156.75 ppm.

In **6** the presence of lactone and acetoxy group in C-3 position were confirmed with the use of ^{13}C-NMR analysis. The signals observed at 178.20 and 96.73 ppm for atoms C-28 and C-13, respectively, indicate the presence of 28,13β-lactone system. The conclusions concerning the acetoxy group were made based on the signals found at 170.67 and 79.98 ppm assigned to atoms C-31 and C-3. In the IR spectrum recorded for **6** two absorption bands at 1775 and 1725 cm^{-1} were found which was similar to the IR spectrum obtained for **5**. The first one can be attributed to C=O group within 28,13β-lactone system and the other one to the acetoxy carbonyl [22]. Moreover, for **6** ^{13}C-NMR spectrum showed resonances at δ 121.37, 13.49 and 50.62 ppm for C-12, C-11 and C-9 of the cyanomethyl group in C-9 position while at δ 150.74 and 116.41 ppm for the new double bond C14=C27. The presence of the double bond between the mentioned atoms indicate structural transformations resulting in the excluding of C-14 atom from ring *D*. In IR spectrum two absorption bands typical for *seco*-nitriles were observed. The first one located at 3060 cm^{-1} was the result of stretching vibrations of hydrogens linked to atom C-27 of the C14=C27 double bond. The other one was situated at 2210 cm^{-1} and indicated the presence of the triple bond within the nitrile group.

In the case of lactam **7** the results of spectral IR, ^{1}H- and ^{13}C-NMR analyses allowed to confirm the formation of lactam system and hydrolysis of 28,13β-lactone ring which led to the regeneration of carboxyl group at C-17 position and formation of new C13=C18 double bond. In the IR spectrum of **7** two absorption bands at 3435 cm^{-1} and 3330 cm^{-1} can be observed. First of them was attributed to the OH group within the COOH function and the other one arose from the NH of lactam system. In ^{1}H-NMR spectrum of **7** only a signal as a doublet situated at 6.69 ppm from the lactam proton was observed. The presence of C13=C18 double bond was confirmed by the signals situated at 135.08 and 134.07 ppm assigned to mentioned atoms. In ^{13}C-NMR spectrum of lactam **7** three characteristic signals for carbonyl C were observed at 180.13, 177.39 and 170.96 ppm. First of them was assigned to C-28 atom, the second one to C-12 and the last one to C-31 atom. Moreover, in the IR spectrum three carbonyl absorption bands were observed at 1730, 1715 and 1655 cm^{-1}. First of them can be assigned to the acetoxy group [22], the second one to the carboxyl function [17] and last one to the lactam system [22].

2.2. X-ray analysis of 5 – 7

X-ray structures of oxime **5**, nitrile **6** and lactam **7** together with the atom numbering schemes are shown in Fig. 1 – 3. The crystal data and refinement parameters are summarized in Table 1.

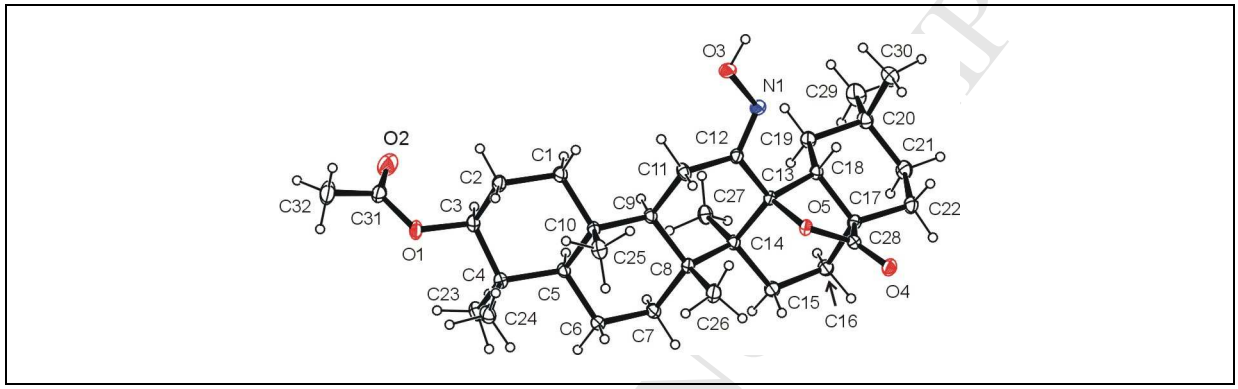


Figure 1. The molecular structure of **5**, showing the atomic labelling scheme. Displacement ellipsoids are drawn at the 50% probability level.

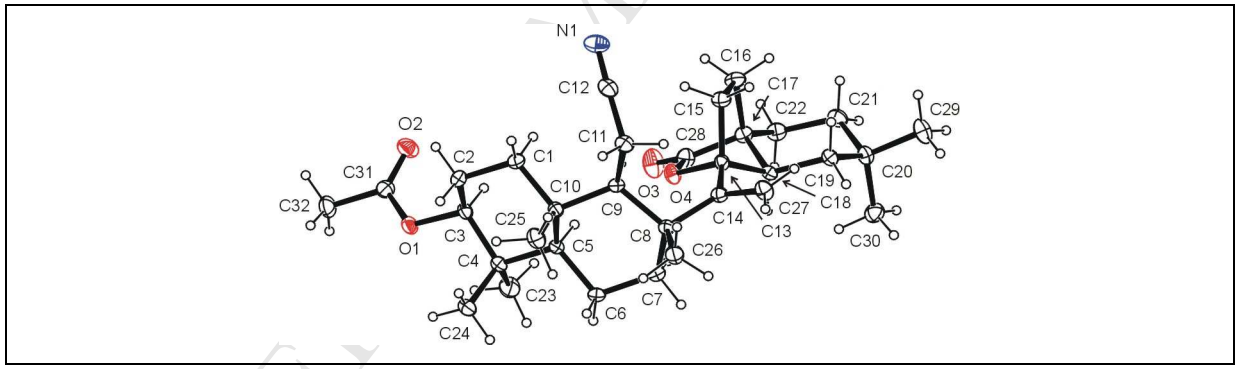


Figure 2. The molecular structure of **6**, showing the atomic labelling scheme. Displacement ellipsoids are drawn at the 50% probability level.

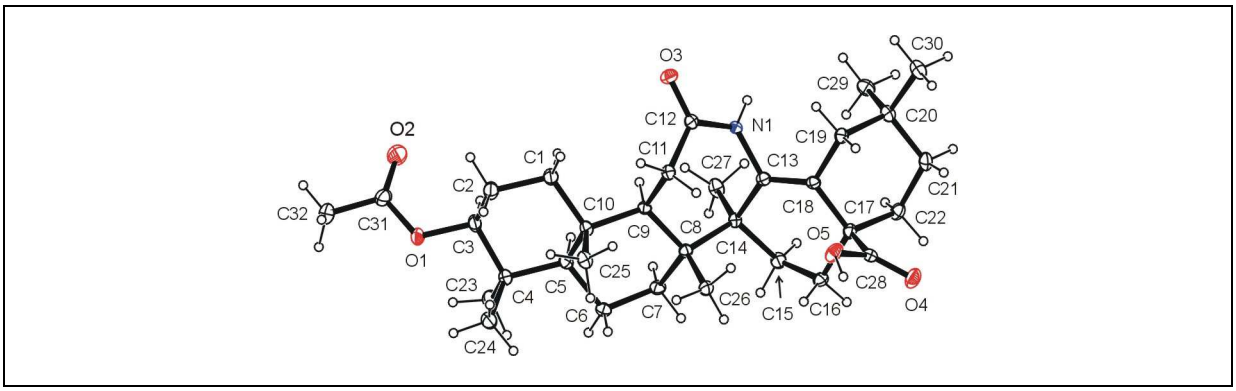


Figure 3. The molecular structure of **7**, showing the atomic labelling scheme. Displacement ellipsoids are drawn at the 50% probability level.

Table 1. Crystal data, data collection and structure refinement for oxime **5**, nitrile **6** and lactam **7**

Compound	oxime **5**	nitrile **6**	lactam **7**
Chemical formula	$C_{32}H_{49}NO_5$	$C_{32}H_{47}NO_4$	$C_{32}H_{49}NO_5$
Formula weight	527.72	509.71	527.72
Temperature [K]	100(2)	100(2)	100(2)
Wavelength [Å]	0.71073	0.71073	0.71073
Crystal system	monoclinic	Triclinic	orthorhombic
Space group	$P2_1$	$P1$	$P2_12_12_1$
Unit cell parameters	a = 7.4690(10) Å b = 15.288(3) Å c = 12.943(3) Å β = 104.78(3)°	a = 8.9322(5) Å b = 9.3534(4)Å c = 10.4867(5) Å α = 102.119(4)° β = 109.856(4)° γ = 111.838(4)°	a = 8.14950 (10) Å b = 14.3252(3) Å c = 25.2958(5) Å
Volume [Å^3]	1429.0(5)	705.88(6)	2953.11(9)
Z	2 (Z'=1)	1 (Z'=1)	4 (Z'=1)
Calculated density [Mg m^{-3}]	1.226	1.199	1.187
Absorption coefficient [mm^{-1}]	0.081	0.077	0.079
F(000)	576	278	1152
Crystal dimensions [mm]	0.58 x 0.22 x 0.15	0.50 x 0.20 x 0.10	0.40 x 0.10 x 0.10
θ Range for data collection [°]	$3.12 \leq \theta \leq 36.85$	$2.54 \leq \theta \leq 29.05°$	$2.63 \leq \theta \leq 29.12$
Max./Min. indices	h: -12 → 11 k: -25 → 25 l: -21 → 21	h: -12 → 12 k: -12 → 12 l: -14 → 14	h: -10 → 10 k: -19 → 15 l: -23 → 33
Reflections collected/unique	25270 / 11246	17497 / 6666	12843 / 6488
Observed reflections [$I \geq 2\sigma(I)$]	10294	6318	5573
Number of parameters	355	341	359
Completeness to θ_{full} = 25.00°	100%	100%	99.9%
Goodness of fit on F^2	1.038	1.086	0.986
$R[I \geq 2\sigma(I)]$	0.0395	0.0292	0.0329
$wR(F^2)$	0.1026	0.0807	0.0764
Largest diff. peak and hole	0.53; -0.27	0.27; -0.16	0.22; -0.19

X-ray analysis revealed that 28,13β-lactone ring was retained only in the product of Beckmann fragmentation (nitrile **6**, Fig. 2). In the molecule of lactam **7** carboxyl group in C17 position and an additional C13=C18 double bond [1.3386(18) Å] in the ring *D* are observed. The carboxyl group is oriented axially with respect to the ring *E* with C28=O4 bond revealing conformation halfway between anticlinal and antiperiplanar [torsion angle C18–C17–C28–O4: -150.40(13)°] with the respect to C17–C18 bond.

X-ray analysis showed that in the molecule of oxime **5** hydroxyimino group in position C(12) of the *C* ring displays *E* configuration [torsion angle O3–N1–C12–C13: -176.48(7)°].

Structural investigation of nitrile **6**, the product of Beckmann fragmentation, showed that the compound was a result of major structural transformations within rings *C* and *D* of the original molecule. The C12–C13 bond was cleaved which resulted in opening of the ring *C* and formation of *β*-oriented cyanomethyl group connected to C9 atom of ring *B*. This group revealed conformation halfway between anticlinal and antiperiplanar (+*ac*/+*ap*) [torsion angle C8–C9–C11–C12: 141.46(9)°] with the respect to C8–C9 bond. The double bond formed along with the nitrile group is located between C14 and C27 atoms [bond length: 1.3337(15) Å]. In the original molecule the first was quaternary carbon atom, while the latter was methyl group. Moreover, the original six-membered *D* ring was transformed into five-membered one which was a result of the cleavage of C14–C15 bond and formation of new C13–C15 bond.

The molecule contains two rigid parts. One of them consists of rings *A* and *B*, whereas the other one consists of rings *D*, *E* and γ-lactone moiety. These two parts are separated by two single bonds, *i.e.* C8–C14 [1.5481(14) Å] and C14–C13 [1.5323(14) Å]. Spatial arrangement of the mentioned systems can be described in terms of mutual orientation of two *trans*-fused systems, *i.e. A* – *B* and *E* – γ-lactone. The dihedral angle between the least squares planes of these two systems assumes 77.39(3)°.

The C14=C27 double bond is anticlinal (+*ac*) with respect to C(8)–C(9) bond [torsion angle C(9)–C(8)–C(14)–C(27): 119.35(11)°] and synclinal (+*sc*) with respect to C(13)–C(18) bond [torsion angle C(18)–C(13)–C(14)–C(27): 58.44(13)°].

In the molecule of **7** seven-membered lactam ring with the N atom connecting C12 and C13 was observed (Fig. 3).

In the molecules of compounds **5** – **7** six-membered saturated rings *A*, *B* (compounds **5** – **7**), *D* (compound **5**) and *E* (compounds **5** and **6**) reveal chair conformation. The named conformation is also observed in the ring *C* of oxime **5** and in the ring *E* of lactam **7**, each of the mentioned rings containing one Csp^2 atom. In the molecule of nitrile **6** five-membered ring *D* displays envelope conformation {puckering parameters [23]: Q = 0.5739(13) Å, φ = 145.12(14)°}. In the molecule of lactam **7** the ring *D* with C13=C18 double bond reveals half-chair conformation [puckering parameters: Q = 0.4194(14) Å, θ = 51.54(19)°, φ = 143.5(2)°]. 28,13β-Lactone rings in compounds **5** and **6** reveal envelope conformation [puckering parameters for oxime **5**: Q = 0.4903(10) Å, φ = 71.18(10)°; for nitrile **6**: Q = 0.5744(13) Å, φ = 73.82(12)°]. Seven-membered lactam ring in the molecule of **7** has chair conformation [puckering parameters: $Q(2)$ = 0.4822(13) Å, $Q(3)$ = 0.6863(13) Å, $\varphi(2)$ = 320.76(16)°, $\varphi(3)$ = 279.33(11)°], despite the presence of two Csp^2 atoms (C-12 and C-13).

In the molecules of all of the investigated compounds planar acetoxy group in C(3) position has β orientation with carbonyl groups displaying conformation halfway between synclinal and anticlinal with the respect to C2–C3 bond (+*sc*/+*ac*) [torsion angle C2–C3···C31–O2 for oxime **5**: 94.16(2)°; for nitrile **6**: 83.37(1)°; for lactam **7**: 78.15(13)°] and similar to synperiplanar with the respect to O1–C3 bond [torsion angle C3–O1–C31–O2 oxime **5**: 17.35(15)°; for nitrile **6**: 4.17(16)°; for lactam **7**: 4.3(2)°].

In the crystal lattices of all of the investigated compounds molecules related by translation along the *a* axis are linked into chains through O–H···O, N–H··O and/or C–H···O hydrogen bonds. In the crystal of oxime **5** molecules are connected by O3–H3···O4^{i} hydrogen bonds [Table 2, Fig. 4] involving hydroxyimino group and carbonyl oxygen atom of lactone system. In the crystal lattice of nitrile **6** molecules are connected by C18–H18B···O2^{i} hydrogen bonds (Table 3, Fig. 5) with carbonyl oxygen of acetoxy group acting as a proton acceptor. In the crystal of lactam **7** molecules are linked through N1–H1···O4^{i} and O5–H5···O3^{ii} hydrogen bonds (Table 4, Fig. 6) involving amide group of lactam system and carboxyl function. The motifs observed in the latter can be described in terms of graph set notation [24-26] as first order chains C(7), C(9) and more interesting second order rings $R_2^2(8)$ (Fig. 7). Additionally, C25–H25A···O2^{ii} hydrogen bonds with carbonyl oxygen atom as a proton acceptor is observed.

Table 2. Hydrogen bond geometry in the crystal lattice of oxime **5**

Hydrogen bond	d(D–H) (Å)	d(H···A) (Å)	d(D····A) (Å)	Angle (D–H···A) (°)
O3–H3···O4^{i}	0.89(2)	1.91(2)	2.758 (12)	159.1(17)

Symmetry code: (i) 1+*x*, *y*, *z*

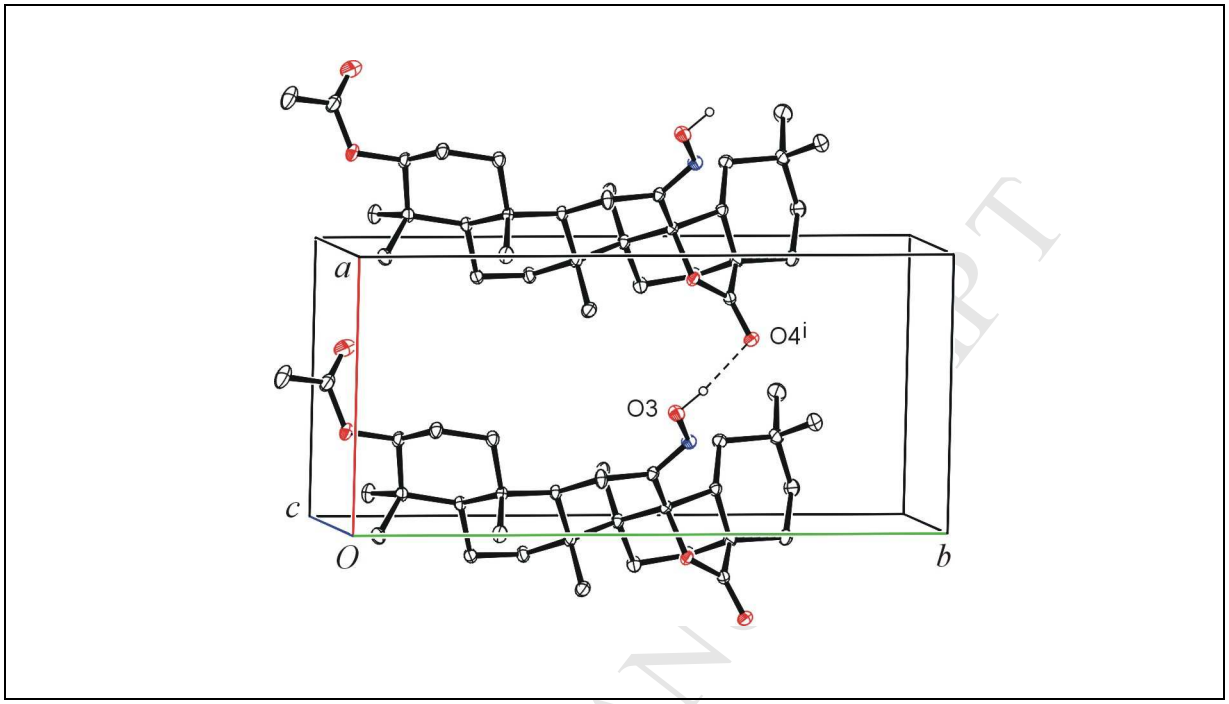


Figure. 4. The hydrogen bonding (dashed lines) in the crystal structure of oxime **5**. The H atoms not involved in hydrogen bonds have been omitted for clarity.

Table 3. Hydrogen bond geometry in the crystal lattice of nitrile **6**.

Hydrogen bond	d(D–H) (Å)	d(H···A) (Å)	d(D····A) (Å)	Angle (D–H···A) (°)
C7–H7A···O4	0.97	2.23	2.9145(14)	127
C9–H9···O4	0.98	2.18	2.9346(15)	133
C18–H18···O2^{i}	0.98	2.46	3.4072(14)	162

Symmetry code: (i) 1+*x*, *y*, *z*.

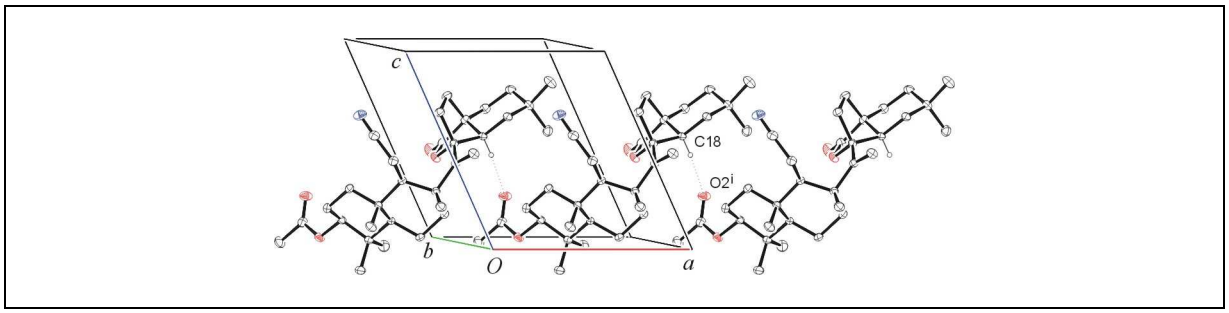


Figure. 5. The hydrogen bonding (dotted lines) in the crystal structure of nitrile **6**. The H atoms not involved in hydrogen bonds have been omitted for clarity.

Table 4. Hydrogen bond geometry in the crystal lattice of lactam **7**.

Hydrogen bond	d(D–H) (Å)	d(H···A) (Å)	d(D····A) (Å)	Angle (D–H···A) (°)
N1–H1···O4^{i}	0.832(16)	2.213(16)	2.9707 (14)	151.4(14)
O5–H5···O3^{ii}	0.94(2)	1.62(2)	2.5503(13)	166.9(19)
C25–H25A···O2^{ii}	0.96	2.56	3.4498(18)	155

Symmetry codes: (i) 1+*x*, *y*, *z*; (ii) -1+*x*, *y*, *z*.

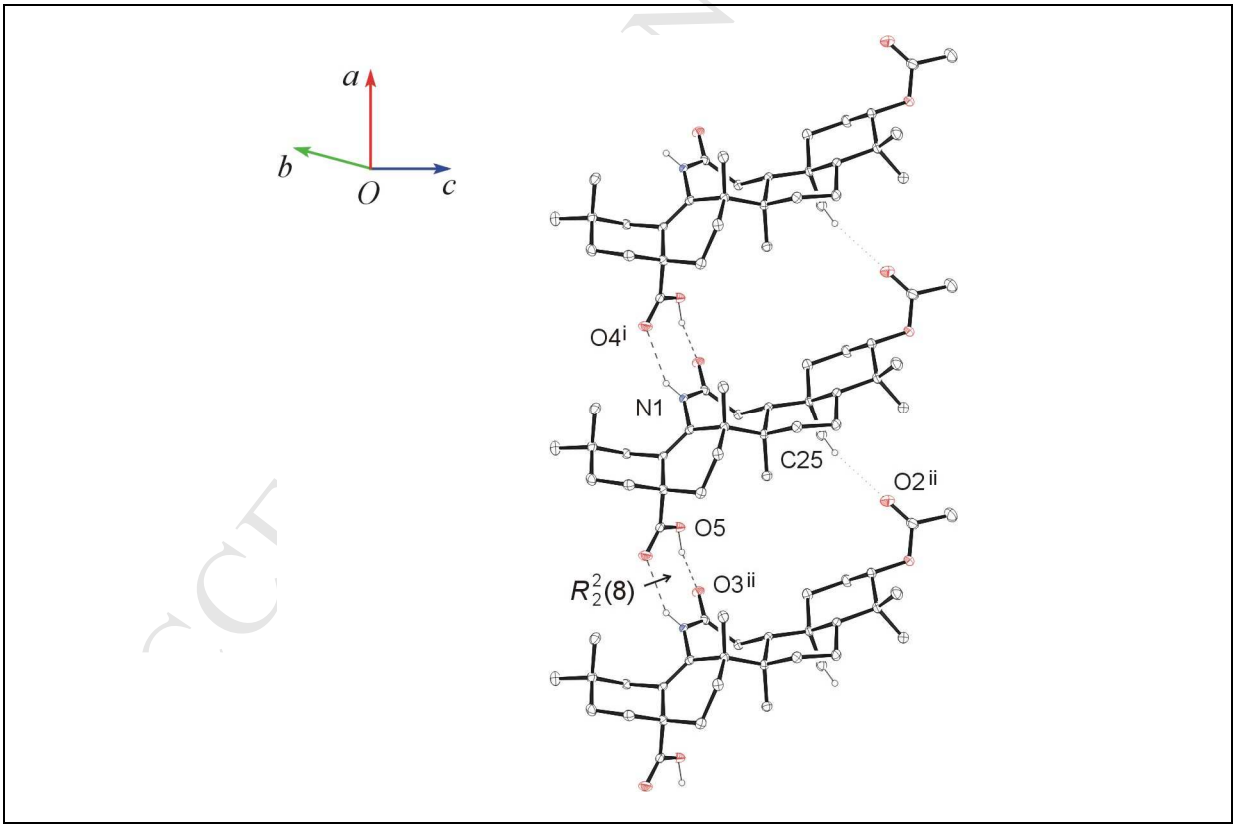
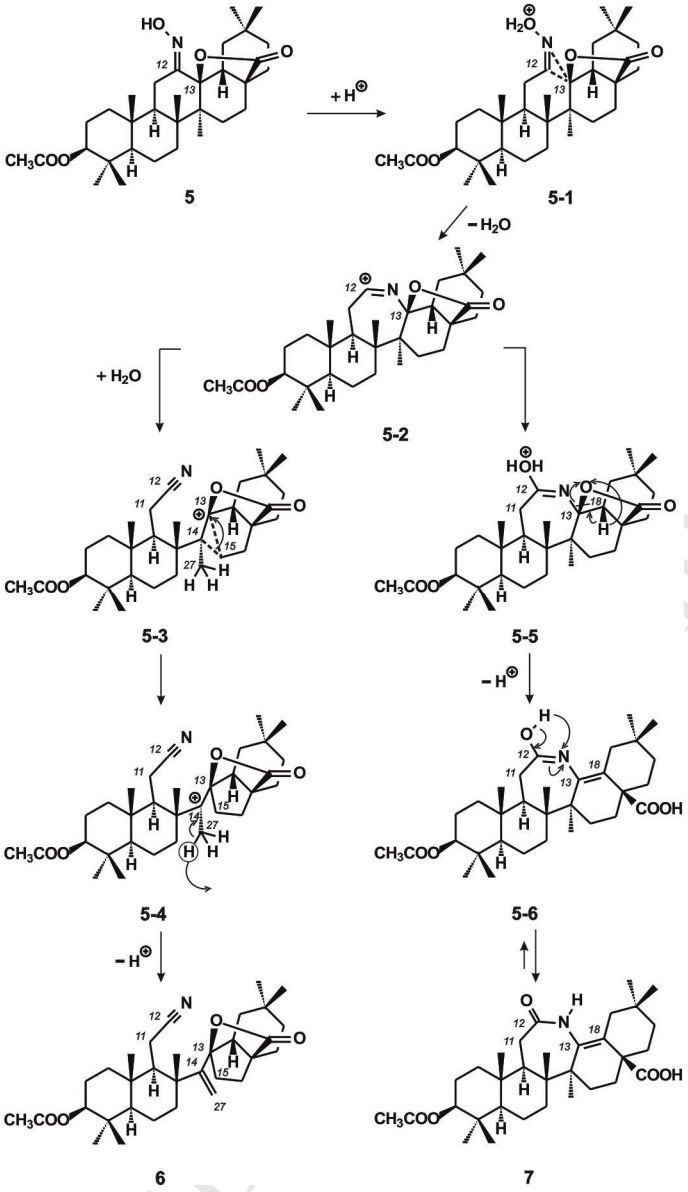


Figure. 6. The hydrogen bonding (dashed and dotted lines) in the crystal structure of lactam **7**. The H atoms not involved in hydrogen bonds have been omitted for clarity.

2.3. Possible reaction mechanism

The structures of substrate **5** and products **6** and **7** of Beckmann rearrangement reaction obtained from X-ray analysis suggest the possible mechanism of the reaction (Scheme 2). The transformation of oxime **5** into nitrile **6** and lactam **7** leads to the formation of nitrilium ion (**5-2**) which is a result of the dissociation of water molecule from the unstable intermediate (**5-1**), cleavage of C12–C13 bond and formation of a new one, *i.e.* C13–N1. The nitrilium ion undergoes two alternative reactions leading to intermediates **5-3** and **5-5**. The first one is the result of the cleavage of C13–N1 bond and the formation of cyanomethyl group comprising of C11, C12 and N1 atoms. The other one is the result of the addition of water molecule to carbocation in C-12 position. Intermediate (**5-3**) is a carbocation with a positive charge localized on C13 atom. Atoms $C13^{+}$, C18 and O3 cannot be arranged in plane which seems to be the reason for 1,2-rearrangement within *D* ring with the contraction of the latter. The C13–C14 bond is cleaved and C13–C15 bond is formed. 1,2-Rearrangement within the ring *D* is followed by the migration of the positive charge from C13 to C14 [intermediate (**5-4**)]. Subsequent dissociation of proton from C27 atom and formation of C14=C27 double bond result in formation of the final product **6**. Intermediate (**5-5**) undergoes two further structural transformations which seem to be independent processes. The first one is proton dissociation from oxonium group in C-12 position, the other one is a hydrolysis of 28,13β-lactone system. As a result of the latter the formation of carboxyl group and C13=C18 double bond in *D* ring are observed (comp. **7**). The reaction route presented in Scheme 2 is based on the assumption that elimination reaction follows commonly accepted *E*1 mechanism. It is noteworthy that lactone system avoided the hydrolysis process in the reaction leading to nitrile **6**. The possible reason for this phenomenon is associated with the presence of the positive charge localized at first on C13 [intermediate (**5-3**)] and then on C14 [intermediate (**5-4**)]. The positive charge may stabilize C13–O3 bond.



Scheme 2. Possible mechanism of transformations leading to nitrile **6** and lactam **7**

3. Conclusions

The molecular structures of 3β-acetoxy-12-hydroxyimino-18β-oleanan-28,13β-olide (**5**) and the products of Beckmann rearrangement, identified as 3β-acetoxy-12-nitrile-12,13-seco-15(14→13)-abeoolean-14(27)-en-28,13β-olide (**6**) and 3β-acetoxy-12-oxo-12a-aza-*C*-homoolean-13(18)-en-28-oic acid (**7**), were elucidated with IR, ^{1}H- and ^{13}C-NMR methods and X-ray analysis. It was revealed that the oxime group in molecule of **5** displayed *E* configuration. Transformation of six-membered isocyclic ring *C* into seven-membered azacyclic one in the molecule of **7** in the course of Beckmann rearrangement was accompanied by the hydrolysis of lactone ring and formation of C13=C18 double bond. In the

molecule of nitrile **6** major structural transformations in comparison with the molecule of original compound **5** were observed. Ring *C* cleavage and nitrile function formation, typical for Beckmann fragmentation course, were followed by 1,2-rearrangement. As a result, in molecule of **6** five-membered ring *D* is observed instead of six-membered one. Double bond is located between C14 and C27. To the best of our knowledge, transformations leading to the ring *D* of oleanane skeleton contraction have never been reported in the literature before.

4. Experimental

4.1. General Experimental Procedures:

The thin layer chromatography (TLC) analyses which were applied in order to evaluate the reactions progress and the purity of compounds were performed on HPTLC aluminium sheets (HPLC Alufolien Kieselgel 60 F245) with benzene and ethyl acetate as a mobile phase. The chromatograms were visualized by spraying them with 10% ethanol solution of sulfuric acid and then keeping the plates in the temperature of about 110^{o}C for a few minutes. The column chromatography was performed using Kieselgel 60; 0.063 – 0.200 mm (70 – 230 mesh). Melting points were determined with open capillary method in Büchi apparatus and were uncorrected. IR spectra were recorded using Specord IR–75 spectrophotometer, for 0.5% mixtures of tested compounds and KBr. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker 600 MHz spectrometer operating at room temperature in deuterated chloroform as a solvent. For ^{1}H-NMR spectra TMS was used as an internal standard. The multiplicities of signals in ^{1}H NMR spectra were marked as follows: s – singlet, dd – doublet of doublets, t – triplet, m – multiplet. Mass spectra (MS) were recorded using AMD 402 spectrophotometer with electroionisation.

4.2. Materials

4.2.1. 3β-Acetoxy-12α-hydroxyoleanan-28,13β-olide (**3**): a saturated solution of acetyloleanolic acid (**2**) (499 mg, 1 mmol) and *m*-CPBA (345 mg, 2 mmol) in chloroform was left in darkness at room temperature for two days. The resulting mixture was washed with 5% solutions: $FeSO_4$, Na_2CO_3, HCl (5 x 10 mL) and next with water (5 x 10 mL). Organic solution was dried with $MgSO_4$ and the solvent was removed with the evaporator. The obtained yellowish, partly crystalline residue was grinded and extracted with ethyl acetate (5 x 10 mL). The extract was rejected and the remaining white residue was dried and crystallized to give the title compound **3**: $C_{32}H_{50}O_5$, mol. mass 514.75; yield: 312 mg (60.6 %); 297 – 300

^{o}C (EtOH) (lit. m.p. 301 – 303 ^{o}C), white needles,; R_f: 0.77 (4:1), 0.31 (9:1), dark-yellow spot; spectral data in accordance with literature data [27].

4.2.2. 3β-Acetoxy-12-oxo-18β-oleanan-28,13β-olide (**4**): the synthesis was performed on the basis of literature protocol [20] starting from 1 mmol (515 mg) of hydroxylactone **3** to give the title compound **4**: $C_{32}H_{48}O_5$; mol. mass: 512.73; yield: 464 mg (90.6 %); m.p.: 287 – 290 ^{o}C (EtOH; decomp), lit. mp.: 287 – 289 ^{o}C [21]; R_f: 0.87 (4:1), 0.67 (9:1), yellow-brown spot; spectral data in accordance with literature data [21].

4.2.3. 3β-Acetoxy-12-hydroxyimino-18β-oleanan-28,13β-olide (**5**): Hydroxylamine hydrochloride (695 mg, 10 mmol) and sodium acetate (1.31 g, 16 mmol) were added to a saturated, hot solution of an appropriate triterpenic ketone (1.02 g, 2 mmol) in ethanol (90 ml) and the obtained mixture was refluxed 90 min, cooled and poured into a 5-fold volume of water. The resulting white precipitate was filtered off, washed with water, dried (yield: 1.00 g, 95.4%) and crystallized from ethanol (small, colourless needles); m.p.: 228 – 231 ^{o}C (decomp.); R_f: 0.79 (4:1), 0.54 (9:1), purple-grey spot. IR (ν, cm^{-1}): 3355 (OH, oxime); 1770 (C=O, lacton); 1730 (C=O, CH_3COO); ^{1}H NMR (δ, ppm): 7.60 (1H, s, oxime); 4.48 (1H, dd, *J* = 5.1 and 11.1, C(3)-H_α); 2.75 (1H, m, C(18)-H_β); 2.05 (3H, s, CH_3COO); 1.20; 0.97; 0.97; 0.95; 0.91; 0.86; 0.86 (7 x 3H, 7 x s, CH_3 groups); ^{13}C NMR (δ, ppm): 179.42 [C_q, C-28]; 171.03 [C_q, CH_3COO]; 156.75 [C_q, C-12]; 90.76 [C_q, C-13]; 80.56 [CH, C-3]; 43.64; 43.30; 42.19; 37.80; 36.98; 31.65 (6 x C_q), 55.03 [CH, C-5]; 48.77 [CH, C-9]; 44.50 [CH, C-18]; 38.30; 37.48; 34.31; 32.91; 27.48; 25.93; 23.49; 20.69; 19.03; 17.45 [10 x CH_2]; 33.26; 27.88; 23.76; 21.27; 18.81; 18.32; 16.38; 15.72 [8 x CH_3]; MS (m/z): 527.7 (0.1%) $M^{+\bullet}$.

4.2.4. 3β-Acetoxy-12-nitrile-12,13-seco-15(14→13)-abeoolean-14(27)-en-28,13β-olide (**6**) and **3β-acetoxy-12-oxo-12a-aza-*C*-homoolean-13(18)-en-28-oic acid** (**7**): $POCl_3$ (0.28 ml, 0.46 g, 3 mmol) was added dropwise to a stirred and cooled solution of oxime **5** (528 mg, 1 mmol) in dried pyridine (12 ml). The resulting solution was left at room temperature overnight. The brownish mixture was then poured into 5-fold volume of water with ice and acidified with HCl. The obtained light-brown precipitate was filtered off, washed with water, dried (502 mg) and subjected to the column chromatography on the silicagel (benzene with ethyl acetate, 4:1 and next ethanol with THF, 1:1).

Compound **6** (nitrile) was obtained from benzene-ethyl acetate fractions (400 mg, 75.7%) and crystallized from ethyl acetate (small, colourless needles); m.p.: 249 – 251 ^{o}C; R_f: 0.68 (4:1), 0.36 (9:1), yellow-brown spot. IR (ν, cm^{-1}): 3060 [C(14)=C(27)]; 2210 (C≡N); 1775 (C=O, lacton); 1725 (C=O, CH_3COO); ^{1}H NMR (δ, ppm): 5.21 [2H, s, C(27)-H_2]; 4.56

[1H, dd, J = 4.5 and 11.7, (C3)-H_α]; 2.45 [1H, t, J = 5.1, C(18)-H_β]; 2.05 (3H, s, CH_3COO); 1.06; 1.01; 0.94; 0.92; 0.89; 0.87 (6 x 3H, 6 x s, C-26, C-30, C-25, C-23, C-29, C-24 methyl groups); ^{13}C NMR (δ, ppm): 178.20 [C_q, C-28]; 170.67 [C_q, $CH_3\underline{CO}O$]; 150.74 [C_q, C-14]; 121.37 [C_q, C-12]; 96.73 [C_q, C-13]; 48.99 [C_q, C-17]; 45.40 [C_q, C-8]; 38.80 [C_q, C-10]; 37.67 [C_q, C-4]; 30.93 [C_q, C-20]; 116.41 [CH_2, C-27]; 13.49 [CH_2, C-11]; 79.98 [CH, C-3]; 53.70 [CH, C-5]; 53.35 [CH, C-18]; 50.62 [CH, C-9]; 38.80; 36.66; 35.45; 34.71; 33.49; 23.68, 23.21; 20.10; 18.18 [9 x CH_2]; 32.83; 27.93; 24.26; 21.27; 21.20; 16.43; 16.09 [7 x CH_3; C-30, C-23, C-29, C-26, $\underline{CH_3}COO$, C-24, C-25]; MS (m/z): 509.4 (12.5%) $M^{+\bullet}$.

Compound **7** (lactam) was obtained from ethanol-THF fractions (108 mg, 20.4%) and crystallized from ethyl acetate (small, colourless needles); m.p.: 278 – 281 ^oC; R_f: 0.21 (AcOEt), pink-brown spot. IR (ν, cm^{-1}): 3435 (O-H, COOH); 3330 (N-H, lactam); 1730 (C=O, CH_3COO); 1715 (C=O, COOH); 1655 (C=O, lactam); ^{1}H NMR (δ, ppm): 7.19 (1H, s, N-H, lactam); 4.51 [1H, dd J = 4.5 and 11.7, C(3)-H_α]; 2.04 (3H, s, $\underline{CH_3}COO$); 1.13; 1.11, 0.95; 0.87; 0.86; 0.84; 0.83 (7 x 3H; 7 x s; C-27, C-26, C-30, C-23, C-25, C-29, C-24 methyl groups); ^{13}C NMR (δ, ppm): 180.13, 177.39; 170.85; 135.03; 134.07; 48.21; 46.63; 44.88; 38.60; 37.75; 32.57; [11 x C_q; C-28, C-12, CH_3COO, C-13, C-18, C-17, C-14, C-8, C-10, C-4, C-20]; 80.28; 54.75; 49.17 [3 x CH, C-3, C-5, C-9]; 40.98; 38.69; 36.21; 35.83; 34.88; 33.05; 32.87; 29.96; 23.36; 18.08 [10 x CH_2, C-19, C-1, C-21, C-16, C-15, C-11, C-22, C-7, C-2, C-6]; 31.76; 27.94; 24.44; 21.90; 21.23; 19.02; 16.63; 16.08 [8 x CH_3, C-30, C-23, C-29, C-27, $\underline{CH_3}COO$, C-26, C-24, C-25]; MS (m/z): 527.3 (40.2%) $M^{+\bullet}$.

4.3. X-ray analysis

Single-crystal X-ray data were collected on Oxford Diffraction KM4 Sapphire (compound **5**) and Oxford Diffraction (Varian Inc.) Xcalibur A (compounds **6** and **7**) diffractometers [28,29] both using graphite-monochromated Mo$K\alpha$ radiation. Intensity data were corrected for the Lorentz and polarization effects. The structure was solved by the direct methods using the program SHELXS-97 [30], and refinement was done against F^2 for all data using SHELXL-97 [31]. All non-hydrogen atoms were refined anisotropically. The positions of the H atoms bonded to N and O atoms were obtained from difference Fourier map and were refined freely. The remaining H atoms were positioned geometrically and were refined using a riding model, with C–H = 0.96 Å (CH_3), 0.97 Å (CH_2), 0.98 Å (Csp^3H), 0.93 Å (Csp^2H) and Uiso(H) = 1.2Ueq(C) or 1.5Ueq(C) for methyl H atoms. The methyl groups were refined as rigid groups, which were allowed to rotate. Software used to prepare materials for

publication was WINGX [32] and PLATON [33]. The molecular illustrations were drawn using ORTEP-3 for Windows [32].

The deposition numbers CCDC953656 for oxime **5**, CCDC953658 for nitrile **6** and CCDC953657 for lactam **7** contain supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html, (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033).

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