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Triterpenoids. Part 21: Oleanolic acid azaderivatives as percutaneous transport promoters

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Abstract—Some new oleanolic acid derivatives with lactame and thiolactame structures in the A- or C-ring were prepared and tested as percutaneous transport promoters in vitro. Their activity was comparable with activity of *N*-dodecylcaprolactame (Azone). A-Thiolactame derivative of methyl oleanolate (**13**) was the most effective compound.

Pentacyclic triterpenes from the amyrine group have been known for a long time to exhibit interesting biological properties. The oleanolic acid derivatives and their antiulceric activity were the most often described.[1] Also, some nitrogen derivatives of the oleanolic acid were recognized as compounds characterized by significant anti-inflammatory and antiulceric activity, sometimes higher than that of the standard Carbenoxolone®.[2] All the pharmacological properties of oleanolic acid were reviewed by Liu,[3] who mentioned also hepatoprotective, anti-neoplastic and anti-hyperlipidemic properties of the acid and emphasized its low toxicity. Recently, many papers have been published, in which new abilities of the oleanolic acid derivatives have been described. Among others, the ability to inhibit the formation of nitrogen oxide in mouse macrophages was reported, especially for 1(2)-unsaturated oleanolic acid derivatives[4] containing additionally in C-2 position an electron withdrawing substituent.[5] Anti-inflammatory activity was also examined, particularly for oleanolic acid oxo-derivatives; this activity lead to inhibition of the complement activity. Cytotoxic and apoptotic activity of the derivatives were also researched.[6] The 3-acyl derivatives of oleanolic acid, particularly of 3-*O*-(3′,3′-dimethyl)-hemisuccinate, were found to exhibit high activity against the HIV-1 virus.[7] Oleanolic and ursolic acids were reported to stabilize liposome membranes.[8] The emulsifying properties of many triterpenoid compounds, the possibility to apply them to cosmetics[3] and their interaction with liposome membranes[8] encouraged us to make an attempt of a synthesis of oleanolic acid derivatives, which may be used as transdermal transport enhancers of other active substances. *N*-Dodecylcaprolactame (Azone®, Laurocapram®) is one of the most effective and well known transepidermal transport enhancer.[9] To perform the synthesis, we decided to introduce to the designed and synthesized triterpene compounds a seven-membered lactame system, which in Azone is considerably responsible for activating transport of other substances through the skin.

To obtain lactame derivatives of oleanolic acid (**1**) (Scheme 1), firstly the substrate was transformed into methyl ester (**2**), which when oxidized with Jones' reagent yielded 3-oxoderivative **6**. A ketone group at C-11 of compound **4** was introduced by allylic oxidation of acetylderivative **3**. Compound **5**, obtained through hydrolysis of compound **4**, was oxidized by Jones' reagent to 3-ketoderivative **8**. Compound **10**, containing a carbonyl group at C-12, was obtained with a yield higher than 80% as a result of oxidation of 3-*O*-acetyl derivative **3**, by using of *m*-chloroperoxybenzoic acid in chloroform and then chromatographic separation on SiO_2 of the obtained mixture of products. In this process the silica gel acts as an acid catalyst and opening the epoxide intermediate to give the appropriate 12-ketone **10**. Of the previously described[10] compounds **6**, **8** and **10**, containing a reactive carbonyl group as well as corresponding oximes **7**, **9** and **11** were obtained, respectively. Under Beckmann rearrangement conditions, with the usage of phosphoryl oxychloride in pyridine,

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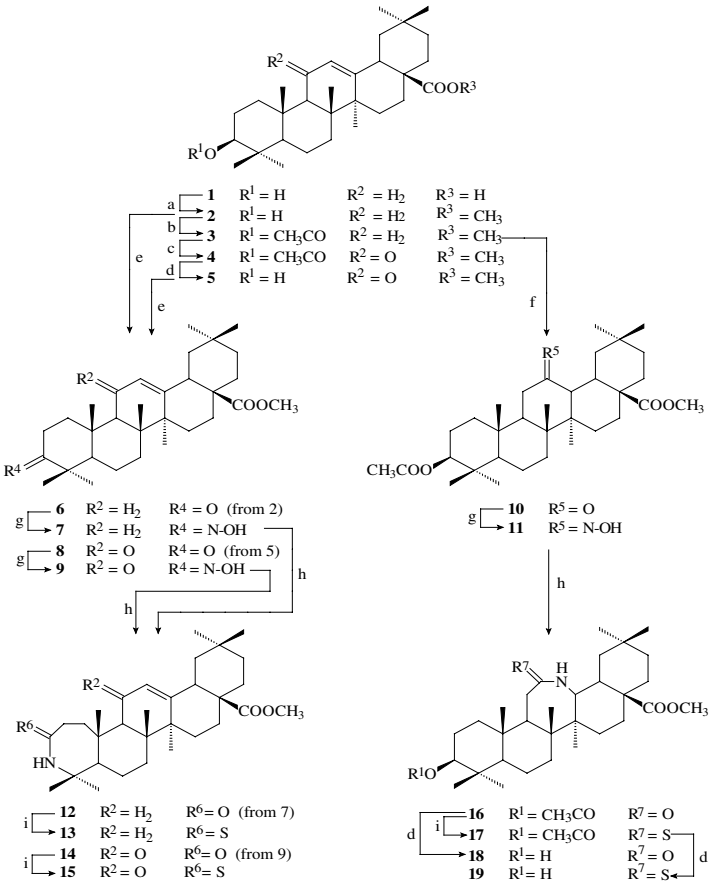

Scheme 1. Reagents and conditions: (a) $(CH_3)_2SO_4$, NaOH in ethanol, ΔT or CH_2N_2 in diethylether; (b) $(CH_3CO)_2O$ in pyridine; (c) *tert*-butyl chromate in CH_2Cl_2 or $Na_2Cr_2O_7$ in CH_3COOH at ca. 60 °C; (d) NaOH in ethanol, ΔT; (e) Jones' reagent in acetone; (f) *m*-CPBA in $CHCl_3$, then SiO_2; (g) $NH_2OH{\cdot}HCl$ in methanol or in pyridine, ΔT; (h) $POCl_3$ in pyridine; (i) Lawesson's reagent in toluene, ΔT.

these oximes were transformed into corresponding lactams **12**[11], **14** and **16** with a seven-membered heterocyclic ring with average yields near to 80%. Similar synthesis of six-membered 23,24-di-nor A-lactame derivatives of oleanolic acid recently was described.[12] Proton magnetic resonance spectra (^{1}H NMR) showed characteristic signals of lactame proton N–H. For compounds **12** and **14**, containing a lactame system in the A-ring and nitrogen atom in 3a-position, this signal was a singlet appeared at 5.69 ppm. For compounds **16** and **18**, containing the lactame system in the C-ring and a nitrogen atom in 12a-position, this signal appeared at 5.53 ppm as a doublet with a coupling constant $J = 5.4$ Hz. The signals observed in the spectra of the carbon magnetic resonance (^{13}C NMR), which coming from a carbonyl group of the lactame system, had a similar position for all the obtained lactams. They appeared, respectively, at 176.9 ppm for compounds **12** and **14** with the lactame system in the A-ring, and at 176.6 ppm for compounds **16** and **18** with the lactame system in the C-ring.[13] Under the action of Lawesson's reagent lactams **12**, **14** and **16** were converted into corresponding thiolactams **13**, **15** and **17**. In the mild alkaline hydrolysis conditions lactame **16** released 3-hydroxyl group, yielding compound **18**. This one, however, as a result of the reaction with Lawesson's reagent, gave a complex mixture of products. Therefore, an appropriate thiolactame **19** was obtained only as a result of hydrolysis of thiolactame **17**.

All the obtained compounds containing lactame (**12**, **14**, **16** and **18**) or thiolactame (**13**, **15**, **17** and **19**) system were examined in vitro to test their ability to activate transport of a therapeutic substance through a model system of synthetic lipophilic membranes stimulating the corneous skin layer. For comparison, analogous tests were carried out for methyl oleanolate (**2**) and for the standard substance—Azone®. The tests were performed with Fürst and co-workers[14] method, with use of five artificial, stabilized, lipophilic membranes system, each of them 8 μm thick, made by using dodecanole as a lipid phase and collodium as a substance to obtain colloxylinic matrix supporting the membranes.[15] Progesterone was chosen as a model substance, for which the degree of penetration was determined. The ointments used in the tests had a form of aqueous-ethanolic gel made on the basis of Carbopol containing 1% of the mentioned model substance, and 0.5%, or—if the solubility allowed—also 1.0%, of the substance tested as a promoter. The ointment was spread on the surface (4 cm^2) of the first membrane of the above mentioned system and left in a thermostat, at 37 ± 0.1 °C for 1 h. After that time, the content of the therapeutic substance was determined separately for each of the five lipophilic

Table 1. Mean quantities of progesterone absorbed from ointment in each membrane expressed as percentage of total progesterone quantity in sample used

No. of promotor and its concentration	Contents of progesterone in each membrane expressed as % of total progesterone in ointment used						Relative increase of progesterone solubility [%]
	Σ1–3	Σ4–5	Σ1–5	Relative contents increase			
				Σ1–3	Σ4–5	Σ1–5	
Without promotor	58.36 ± 4.65	12.11 ± 4.57	70.47 ± 2.26	—	—	—	—
Azone (0.5%)	77.37 ± 3.70	10.74 ± 2.03	88.11 ± 4.40	+32.6	−11.3	+25.0	+1.08
2 (0.5%)	53.04 ± 1.64	13.22 ± 0.46	66.26 ± 1.57	−9.1	+9.2	−6.0	+1.92
12 (0.5%)	66.71 ± 2.73	11.27 ± 2.17	77.99 ± 4.21	+14.3	−6.9	+10.7	+2.77
13 (0.5%)	75.71 ± 4.05	17.71 ± 4.66	93.42 ± 4.14	+29.7	+46.2	+32.6	−0.98
14 (0.5%)	69.29 ± 4.89	12.88 ± 1.70	82.17 ± 4.21	+18.7	+6.4	+16.6	+2.12
15 (0.5%)	75.31 ± 4.62	15.34 ± 4.32	90.65 ± 4.98	+29.0	+26.7	+28.6	−1.44
15 (1.0%)	74.41 ± 4.35	19.95 ± 4.37	94.36 ± 4.86	+27.5	+64.7	+33.9	−1.77
16 (0.5%)	65.31 ± 5.05	12.97 ± 4.54	78.28 ± 4.47	+11.9	+7.1	+11.1	+2.77
16 (1.0%)	71.87 ± 5.48	15.92 ± 4.64	87.79 ± 4.61	+23.1	+31.5	+24.6	+4.08
17 (0.5%)	63.00 ± 4.71	13.64 ± 4.34	76.64 ± 4.49	+8.0	+12.6	+8.8	−0.99
17 (1.0%)	67.51 ± 4.13	16.57 ± 2.82	84.08 ± 4.95	+15.7	+36.8	+19.6	−0.91
18 (0.5%)	60.78 ± 4.30	14.14 ± 4.24	74.92 ± 3.87	+4.1	+16.8	+6.3	+3.02
19 (0.5%)	62.65 ± 2.98	14.16 ± 3.12	76.81 ± 3.36	+7.4	+16.9	+9.0	–0.84

membranes. To determine the content, each of the separated membranes was dissolved separately in 3 cm^3 of methanol. Subsequently to thus obtained solutions 0.5 cm^3 of isonicotinic acid hydrazide solution was added to trigger a colour reaction and then the content of progesterone was tested using the UV spectrophotometric method ($\lambda = 395$ nm). For statistical analysis of the results, each measurement was taken eight times. This allowed estimating the total amount of the medicine absorbed in the presence of the promoter as well as the influence of the promoter on the degree of penetration of the medicinal substance in successive membranes imitating the corneous skin layer. For each of the promoters the content of progesterone was added up separately for membranes 1–3 and 4–5, as well as for all membranes 1–5. In order to evaluate a potential dependence of the ability to activate the transepidermal transport on the properties of the tested substance, also the effect of the tested promoter on the solubility of progesterone in the system of solvents used in the ointments preparing was studied.

On the basis of the results of the tests (Table 1) the following conclusions can be drawn: the influence of the studied substances on progesterone solubility is slight; positive for lactams and negative for thiolactams. All compounds containing the lactame or thiolactame structure lead to a significant increase in the amount of progesterone absorbed in the system of lipophilic membranes. This effect has not been observed for oleanolic acid methyl ester (**2**). The degree of increase in the absorption of the model substance changes, depending on the structure of promoter and its concentration. The highest increase in the amount of the absorbed substance was reported in the presence of thiolactams **13** and **15**, containing the heterocyclic system in the A-ring. In the presence of these compounds the highest increase in progesterone concentration in membranes No. 4 and 5 was observed, which is indication of their favourable effect on the degree and depth of medicine penetration. The effect of the ketone group at C-11 in compounds **14** and **15** on the promoter activity is small. In a group of compounds with the modified C-ring also thiolactams **17** and **19** were more active than the corresponding lactams **16** and **18**. Compounds with an acetoxy group in 3β position (**16** and **17**) were more active than the corresponding compounds with a free 3β-OH group (**18** and **19**). Increase in the concentration of the studied compounds from 0.5% to 1.0% causes the increase in the observed accelerating effect, yet it is disproportional to the increase in their concentration.

Of all the tested methyl oleanolate azaderivatives, compound **13** with the seven-membered thiolactame in the A-ring appeared to be the most effective promoter.

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13. Selected spectral data for exemplify compounds. **12**: white needles, mp 293–294 °C; IR (KBr) 3220, 1725, 1680 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.69 (s, 1H), 5.29 (t, 1H, $J = 3.4$ Hz), 3.63 (s, 3H), 2.93 (dd, 1H, $J = 13.8$, 4.0 Hz), 2.75 (m, 1H), 2.46 (m, 2H), 2.05 (td, 1H, $J = 13.8$, 4.0 Hz), 1.77–0.76 (many m with seven s of CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 178.2, 176.9, 143.6, 122.3, 56.4, 55.7, 51.5, 47.2, 46.7, 45.7, 41.8, 41.2, 40.9, 39.4, 37.0, 34.6, 33.8, 33.0, 32.4, 32.3, 31.5, 30.6, 27.6, 26.3, 25.6, 23.8, 23.6, 23.0, 21.7, 16.9,16.2; MS (EI): 483.4, 424.4, 262.2, 203.2; HR-MS: calcd for $C_{31}H_{49}NO_3$[M]: 483.3712; found: 483.3741. **16**: white needles, mp 287–289 °C; IR (KBr) 3410, 1720, 1670 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.53 (d, 1H, $J = 5.4$ Hz), 4.49 (dd, 1H, $J = 11.4$, 5.0 Hz), 4.01 (t, 1H, $J = 6.0$ Hz), 3.73 (s, 3H), 2.51 (dt, 1H, $J = 13.6$, 5.0 Hz), 2.44 (dd, 1H, $J = 13.6$, 10.4 Hz), 2.04 (s, 3H), 2.10–0.75 (many m with seven s of CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 177.3, 176.6, 170.6, 80.2, 54.4, 52.0, 50.4, 48.6, 47.3, 45.1, 42.7, 38.6, 38.5, 37.8, 36.1, 35.9, 34.8, 33.6, 33.4 (double signal), 32.7, 30.9, 28.4, 28.0, 23.6, 23.4, 21.8, 21.3, 17.9, 17.6, 17.0, 16.7, 15.9; MS (EI): 543.0, 484.1, 308.1, 248.2; HR-MS: calcd for $C_{33}H_{53}NO_5$[M]: 543.3924; found: 543.3908.
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