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Revision and Confirmation of the Regiochemistry of Isoxazoles Derived from Methyl Oleanonate and Lanost-8-en-3-one. Synthesis of a New Lanostane Triterpenoid with a Cyano-enone Functionality in Ring A

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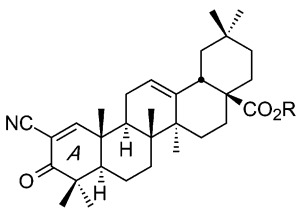
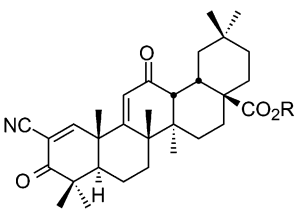
Abstract: It was previously reported that methyl oleanonate (**5**) and lanost-8-en-3-one (**10**) give predominantly [3,2-*c*]isoxazoles. On the contrary, we have confirmed that both compounds **5** and **10** do not give [3,2-*c*]isoxazoles but rather afford regioselectively [2,3-*d*]isoxazoles in good yields. Consequently, a new lanostane triterpenoid with a cyano-enone functionality in ring A was synthesized in two steps from the corresponding [2,3-*d*]isoxazole, which is interesting from the perspective of biological activity because lanosterol is the biogenetic precursor of steroids.

Triterpenoids are natural products with 30 carbon atoms, biosynthetically derived from the cyclization of squalene.[1] Many triterpenoids are reported to have various interesting biological, pharmacological, or medicinal activities including antiinflammatory and anticarcinogenic activities.[2] However, in many cases, the potency of these triterpenoids is relatively weak. To discover new antiinflammatory and cancer chemopreventive drugs from triterpenoids, we have synthesized and biologically evaluated over 270 derivatives of oleanolic and ursolic acids, commercially available naturally occurring triterpenoids.[3] In these investigations, we have found that a cyano-enone functionality in ring A (e.g., compounds **1**−**4**) is very important to exhibit high



1 R = Me
2 R = H



3 R = Me
4 R = H

inhibitory activity against nitric oxide production induced by interferon-γ in mouse macrophages.[3b−e] In connection with the construction of a cyano-enone functionality in ring A, we have found that the regiochemistry of isoxazoles **7** and **12** derived from methyl oleanonate (**5**)[4] and lanost-8-en-3-one (**10**)[5] is different from the one which was previously assigned.[6−8] We report here the revision and confirmation of the regiochemistry of these isoxazoles and subsequent synthesis of a new lanostane triterpenoid **14** with a cyano-enone functionality in ring A, which is interesting from the perspective of biological activity because the triterpene lanosterol is the biogenetic precursor of steroids.

Results and Discussion

We previously reported that biologically active oleanolic acid derivative **1** with a cyano-enone functionality in ring A is synthesized in four steps from methyl oleanonate (**5**) according to the synthetic route shown in Scheme 1.[3c,d] Because isoxazole **7**, which is regioselectively synthesized in 86% yield by condensation of **6** with hydroxylamine hydrochloride in aqueous EtOH (reflux) (our method), gives **1** via a mixture of **8a**−**c** in 87% yield, we assigned isoxazole **7** as the [2,3-*d*]isoxazole.[3c,d,9] However, our literature survey disclosed that two articles[6,7] reported that this isoxazole, synthesized from the same hydroxymethylene **6** as for **7**, is actually the isomeric [3,2-*c*]isoxazole **9**.[9] The authors of both articles assign this regiochemistry to their isoxazole because treatment of their isoxazole with sodium methoxide in THF did not give α-cyano-ketone **8b**,**c**. Both authors used Briggs's method[8] for the synthesis of their isoxazoles, which is different from our method. Briggs and co-workers synthesized [3,2-*c*]isoxazole **15** as a major product by con-

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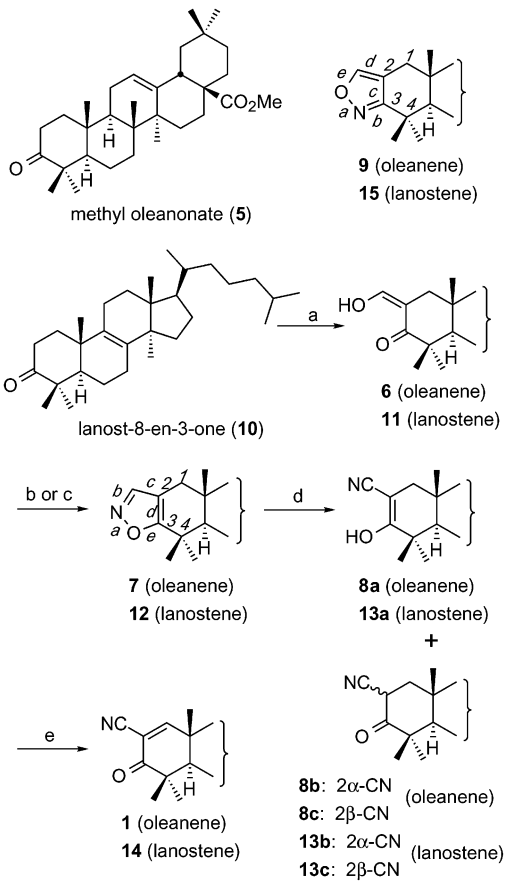
(9) Throughout this article (except for the Experimental Section), for the ease of comparison, the same atom numbering as used with the isoxazoles derived from lanost-8-en-3-one is used for the isoxazoles derived from methyl oleanonate.

SCHEME 1[a]



[a] Key: (a) HCO_2Et, NaOMe, PhH; (b) $NH_2OH{\cdot}HCl$, aqueous EtOH (our method); (c) $NH_2OH{\cdot}HCl$, NaOAc, aqueous AcOH (Briggs's method); (d) NaOMe, MeOH, Et_2O; (e) DDQ, PhH.

densation of **11** with hydroxylamine hydrochloride in the presence of sodium acetate in aqueous acetic acid (at 60 °C). Therefore, because we wondered if Briggs's method gives a different isoxazole, we intended to synthesize the isoxazole from **6** using this method. Briggs's method gave an isoxazole as a single compound from **6** (yield, 79%), and this isoxazole was identical with isoxazole **7** by TLC, UV, IR, ^{1}H and ^{13}C NMR, and MS spectra. From these results, we have confirmed that condensation of hydroxymethylene **6** with hydroxylamine hydrochloride does not give [3,2-*c*]isoxazole **9**, but rather gives [2,3-*d*]-isoxazole **7** predominantly.

Manson and co-workers reported that 17β-hydroxy-4,4-dimethylandrostan-3-one gives [2,3-*d*]isoxazole exclusively,[10] of which ring A has the same structure as those of methyl oleanonate (**5**) and lanost-8-en-3-one (**10**). At this point, only Briggs's result was inconsistent with Manson's and our results. Because the 4,4-dimethyl group sterically hinders the nucleophilic attack of hydroxylamine at the 3-position, the formation of [2,3-*d*]-isoxazole seems to be reasonable rather than that of [3,2-*c*]isoxazole. Therefore, we decided to examine the regiochemistry of [3,2-*c*]isoxazole **15** that Briggs reported. For this purpose, we synthesized the alleged isoxazole **15** from lanost-8-en-3-one (**10**) according to Briggs's synthetic route (Scheme 1). Formylation of **10** with ethyl formate in the presence of sodium methoxide in benzene[11,12] gave hydroxymethylene **11** in 94% yield. Because a hydroxy proton and the vicinal olefin proton were observed at 14.94 ppm (1H, d, J = 2.9 Hz) and 8.69 ppm (1H, d, J = 2.9 Hz) in the ^{1}H NMR spectrum ($CDCl_3$), **11** was shown to have the enolic 2-hydroxymethylene form. Briggs's and our methods gave the same isoxazoles **12** from **11** in the same yield (89%). Cleavage of the isoxazole moiety of **12** with sodium methoxide[13] gave inseparable nitriles **13a**−**c** in 93% yield. The IR spectrum (KBr) showed a characteristic absorption of a nitrile group at 2213 cm^{-1}. However, the ^{1}H and ^{13}C NMR gave uninterpretable spectra which suggest that this compound is a mixture of three tautomers in $CDCl_3$. For the confirmation of the regiochemistry of isoxazole **12**, this mixture was oxidized with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to give cyano-enone **14** in 72% yield as a single compound. This structure was fully characterized by IR, UV, ^{1}H and ^{13}C NMR, low and high MS spectra, and elemental analysis (see Experimental Section). Because isoxazole **12** gave cyano-enone **14** via **13a**−**c**, the regiochemistry of the isoxazole moiety was assigned as the [2,3-*d*]isoxazole. From these results, we have confirmed that condensation of hydroxymethylene **11** with hydroxylamine hydrochloride does not give [3,2-*c*]isoxazole **15** but [2,3-*d*]isoxazole **12** regioselectively.

In conclusion, it has been confirmed that methyl oleanonate (**5**) and lanost-8-en-3-one (**10**) give the corresponding [2,3-*d*]isoxazoles predominantly. This result is consistent with Manson's result that 4,4-dimethylandrostan-3-one gives [2,3-*d*]isoxazole. Also, the first synthesis of a new lanostane triterpenoid **14** with a cyano-enone functionality in ring A, which is interesting from the perspective of biological activity, was achieved. Studies on the biological properties of this compound are in progress.

Experimental Section

General. All experiments were performed under N_2 atmosphere. The standard workup method was as follows: an organic extract was washed with saturated aqueous $NaHCO_3$ solution (three times) and then saturated aqueous NaCl solution (three times), dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a compound.

Methyl Isoxazolo[4,5-*b*]olean-12-en-28-oate (7). To a suspension of methyl 2-(hydroxymethylene)-3-oxoolean-12-en-28-oate (**6**)[3d] (50 mg, 0.1 mmol) in acetic acid (2.5 mL) was added a solution of sodium acetate (25 mg, 0.3 mmol) and hydroxylamine hydrochloride (25 mg, 0.36 mmol) in water (0.5 mL). The mixture was heated at 60 °C for 1 h. The mixture was poured into water. The aqueous mixture was extracted with EtOAc three times. The extract was worked up according to the standard method to give a solid (51 mg). The solid was subjected to flash column chromatography [hexanes/EtOAc (5:1)] to give **7** as an amor-

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(12) Briggs prepared the hydroxymethylene **11** from **10** using NaH instead of sodium methoxide.[8] We confirmed that both hydroxymethylenes are the same by TLC, UV, IR, ^{1}H and ^{13}C NMR, and MS spectra.

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phous solid (40 mg, 80%). The solid was recrystallized from MeOH to give colorless needles: mp 193.4−194.1 °C (lit.,[6] 193−195 °C). This isoxazole was identical with the isoxazole[3d] prepared by our method by mp, TLC, UV, IR, ^{1}H and ^{13}C NMR, and MS spectra.

2-(Hydroxymethylene)lanost-8-en-3-one (11). To a stirred mixture of lanost-8-en-3-one (**10**)[14] (310 mg, 0.73 mmol) and ethyl formate (97%) (262 mg, 3.4 mmol) in benzene (7.3 mL) was added sodium methoxide (182 mg, 3.4 mmol). The mixture was stirred at room temperature for 1 h. After the mixture was washed with 5% aqueous HCl solution twice, it was worked up according to the standard method to give **11** as colorless needles (313 mg, 94%): mp 124.1−124.5 °C (lit.,[8] 125.5−127 °C); $[\alpha]^{27}_D$ +130° (*c* 1.05, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 296 (4.07) nm; IR (KBr) 2957, 2867, 1638, 1591, 1468 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 14.94 (1H, d, J = 2.9 Hz), 8.69 (1H, d, J = 2.9 Hz), 2.38 (1H, d, J = 14.3 Hz), 1.21, 1.15, 0.97, 0.91 (each 3H, s), 0.91 (3H, d, J = 6.2 Hz), 0.880 (3H, d, J = 6.6 Hz), 0.875 (3H, d, J = 6.6 Hz), 0.73 (3H, s); ^{13}C NMR ($CDCl_3$) δ 190.7, 189.4, 136.3, 131.9, 106.3, 50.7, 50.3, 48.7, 44.6, 40.5, 39.7, 36.8, 36.70, 36.66, 36.6, 31.21, 31.17, 28.7, 28.4, 28.2, 26.5, 24.5, 24.3, 23.1, 22.8, 21.1, 20.8, 19.6, 18.9, 17.8, 16.2; EIMS (70 eV) *m*/*z* [M]$^+$ 454 (33), 439 (100), 328 (32), 275 (12); HREIMS Calcd for $C_{31}H_{50}O_2$: 454.3811, Found: 454.3809. Anal. Calcd for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 81.66; H, 11.18.

Lanost-8-eno[2,3-*d*]isoxazole (12). By our method: A mixture of **11** (100 mg, 0.22 mmol), hydroxylamine hydrochloride (151 mg, 2.2 mmol) in water (0.21 mL), and EtOH (5.2 mL) was heated under reflux for 1 h. After EtOH was removed in vacuo, a mixture of CH_2Cl_2 and Et_2O (1:2) was added to the resultant mixture. The organic layer was worked up according to the standard method to give a solid (98 mg). The solid was subjected to flash column chromatography [hexanes/EtOAc (10:1)] to give **12** as colorless needles (88 mg, 89%): mp 140.5−141.5 °C; $[\alpha]^{27}_D$ +111° (*c* 0.25, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 229 (3.86) nm; IR (KBr) 2953, 2878, 1630, 1478, 1461 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.02 (1H, s), 2.51 (1H, d, J = 15.0 Hz), 2.21 (1H, d, J = 15.0 Hz), 1.31, 1.24, 0.95, 0.92(each 3H, s), 0.91 (3H, d, J = 5.9 Hz), 0.88 (3H, d, J = 6.6 Hz), 0.87 (3H, d, J = 6.6 Hz), 0.73 (3H, s); ^{13}C NMR ($CDCl_3$) δ 173.6, 150.5, 136.3, 131.9, 109.7, 50.7, 50.3, 44.6, 39.7, 39.0, 36.7, 36.6, 35.0, 32.7, 31.24, 31.19, 29.0, 28.3, 28.2, 26.7, 24.5, 24.3, 23.0, 22.8, 21.3, 21.1, 19.0, 18.9, 18.7, 16.2; EIMS (70 eV) *m*/*z* 451 [M]$^+$ (24), 436 (100), 328 (21), 282 (15), 270 (19); HREIMS Calcd for $C_{31}H_{49}NO$: 451.3814, Found: 451.3815. Anal. Calcd for $C_{31}H_{49}NO$: C, 82.42; H, 10.98; N 3.10. Found: C, 82.34; H, 10.96; N 3.24.

By Briggs's method: A solution of hydroxylamine hydrochloride (16 mg, 0.23 mmol) and sodium acetate (37 mg, 0.45 mmol) in water (0.1 mL) was added dropwise during 10 min to a solution of **11** (100 mg, 0.22 mmol) in acetic acid (3.4 mL) at 60 °C. The mixture was stirred at 60 °C for 1 h. The reaction mixture was poured into water, and the precipitate was extracted with Et_2O. The extract was worked up by the standard method to give **12** as colorless needles (87 mg, 89%). This isoxazole was identical with the isoxazole prepared according to our method (vide supra) by TLC, UV, IR, ^{1}H and ^{13}C NMR, and MS spectra.

3-Oxolanosta-1,8-diene-2-carbonitrile (14). To a solution of sodium methoxide (310 mg, 5.7 mmol) in dry MeOH (3.5 mL) was added a solution of **12** (80 mg, 0.18 mmol) in dry Et_2O (4.1 mL). The mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with a mixture of CH_2Cl_2 and Et_2O (1:2) (20 mL). After the mixture was washed with 5% aqueous HCl solution (twice), it was worked up by the standard method to give 3-oxolanost-8-en-2-carbonitrile (**13**) as a colorless solid (74 mg, 93%): IR (KBr) 3256, 2964, 2878, 2213, 1722, 1626, 1467 cm^{-1}; EIMS (70 eV) *m*/*z* 451 [M]$^+$ (21), 436 (100), 296 (10), 270 (26); HREIMS Calcd for $C_{31}H_{49}NO$: 451.3814, Found: 451.3812. A mixture of **13** (64 mg, 0.14 mmol) and DDQ (98%) (45 mg, 0.19 mmol) in dry benzene (5.7 mL) was heated under reflux for 10 min. After removal of insoluble matter by filtration, the filtrate was evaporated in vacuo to give a brown solid (109 mg). The solid was purified by flash column chromatography [hexanes/EtOAc (7:1)], followed by prep-TLC [hexanes/EtOAc (7:1)] to give **14** as colorless needles (45.6 mg, 72%): mp 131.5−131.9 °C; $[\alpha]^{27}_D$ +6.0° (*c* 1.0, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 236 (3.85) nm; IR (KBr) 2950, 2889, 2836, 2233, 1685, 1611, 1468 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.02 (1H, s), 2.20 (4H, m), 1.31, 1.23, 1.13 (each 3H, s), 0.92 (3H, d, J = 6.2 Hz), 0.89 (3H, s), 0.88 (3H, d, J = 6.6 Hz), 0.87 (3H, d, J = 6.6 Hz), 0.72 (3H, s); ^{13}C NMR ($CDCl_3$) δ 198.2, 168.7, 138.5, 129.1, 115.4, 114.7, 50.6, 50.3, 47.0, 44.9, 44.7, 41.9, 39.7, 36.6, 30.9, 28.3, 28.2, 26.4, 25.4, 24.3, 24.2, 23.0, 22.9, 22.8, 22.7, 21.4, 18.9, 18.3, 16.1; EIMS (70 eV) *m*/*z* 449 [M]$^+$ (17), 434 (100), 336 (15), 294 (12), 280 (32), 268 (36); HREIMS Calcd for $C_{31}H_{47}NO$: 449.3658, Found: 449.3658. Anal. Calcd for $C_{31}H_{47}NO \cdot 1/5H_2O$: C, 82.14; H, 10.54; N, 3.09. Found: C, 82.23; H, 10.54; N, 3.10.

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(14) Lanost-8-en-3-one (**10**) was synthesized by Jones oxidation of lanost-8-en-3β-ol,[8] which was purified from a commercially available mixture of lanost-8-en-3β-ol and lanosta-8,24-dien-3β-ol (ratio: 40:60) (Aldrich Chemical Co.) as follows: This mixture was acetylated with Ac_2O and pyridine, followed by oxidation with *m*CPBA, to give a mixture of lanost-8-en-3β-yl acetate and 24,25-epoxylanost-8-en-3β-yl acetate, which was easily separated by flash column chromatography [hexanes/EtOAc (10:1)], followed by hydrolysis with KOH in MeOH (reflux) to give pure lanost-8-en-3β-ol, mp 144−144.5 °C (lit.,[5] mp 144−145 °C).