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A Spontaneous Conversion of Gagamine, a Steroidal Alkaloid, into Lineolon *via* Internal Acyl Migrations and the Following Elimination

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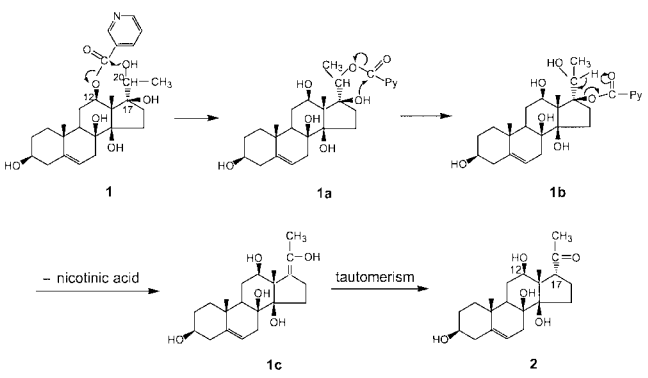
Gagamine (**1**) is a new steroidal alkaloid isolated by us from the Japanese medicinal asclepiadaceae plant *Cynanchum caudatum*.[1] This compound is a 12-*O*-nicotinoyl ester of sarcostin, a polyoxypregnane steroid. Internal acyl migration in naturally occurring pregnane esters has been reported: the acyl migration in wilforine (12-*O*-cinnamoyl-20-*O*-ikemaoylsarcostin),[2] gagaminine (12-*O*-cinnamoyl-20-*O*-nicotinoylsarcostin),[3] dehydrotomentosin (12-*O*-tigloyl-20-*O*-acetylsarcostin),[4] and gymnemarsgenin (12-*O*-cinnamoyl-20-*O*-benzoylsarcostin)[5] underwent from the hydroxyl group of C-12 to C-20 under mild alkaline hydrolysis conditions. We now demonstrate the spontaneous acyl migration of gagamine without alkaline conditions and discuss its tentative mechanism.

Gagamine (**1**) was recrystallized in MeOH several times to give colorless amorphous solid. This compound unexpectedly showed a negative Meyer reaction, suggesting not an alkaloid but revealed a positive Liebermann-Burchard test, indicating a steroid. To elucidate the structure of this unknown steroid, its physical and spectral properties were measured. Its melting point (240-242 °C, dec.) was very different from that of gagamine (mp. 168-170 °C) and IR spectrum contains a carbonyl peak at 1684 cm^{-1} being an absorption of keto group instead of ester group (1718 cm^{-1}) for gagamine. The extremely low frequency for the unconjugated ketone is probably due to intra- and inter-hydrogen bondings and are also observed with other hydroxylated pregnane derivatives, *e.g.*, 1680 cm^{-1} for fukujusone.[6] The 21 carbon signals in the ^{13}C-NMR spectrum were characterized by a DEPT experiment, which shows that this compound has a pregnane skeleton having three methyls, seven methylenes, five methines, and five quaternary carbons including one carbonyl carbon (δ 214.81). In addition, the ^1H-NMR spectrum of this pregnanes exhibits no aromatic proton signals for a nicotinoyl group and lacks OH-17 and OH-20 signals, but showed a OH-12 signal (D_2O exchange) with an additional methine signal (δ 3.40, t, J (16α,17) = 11.1 Hz; J (16β,17) = 9.3 Hz), which could be assigned as a H-17 proton instead of a OH-17 proton in gagamine.

Above spectral data, CD spectrum (negative Cotton effect; $\Delta\varepsilon = -1.20$ at 283 nm) and the comparison of MS data reported[7] indicated this compound to be lineolon. Lineolon (deacylcynanchogenin) has been known to be isolated (as an aglycone) from natural sources[7-9] or by synthesis from sarcostin with Serini reaction.[10] Moreover, alkaline hydrolysis of some 12-*O*-esters[6] yielded the mixture of lineolon (17α form) and isolineolon (17β form), which have been reported



Scheme 1. A tentative mechanism for the internal acyl migration of gagamine (1).

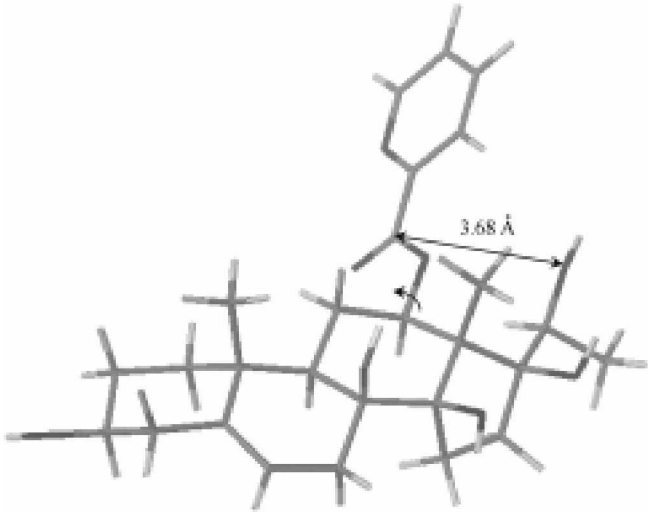


Figure 1. A steric view of gagamine (1) by computer modeling.

to be distinguished from each other by MS,[7] NMR[11] and ORD spectra.[12]

As introduced above, the acyl migration from C-12 to C-20 occurred in C-12, C-20 di-*O*-acyl esters under mild alkaline hydrolysis conditions.[2-5] Gagamine (C-12-*O*-nicotinoyl ester), however, directly afforded lineolon by elimination of the nicotinoyl group[13] without any reagent. The tentative reaction mechanism (Scheme 1) is proposed to explain this unusual observation. As shown in Figure 1, the molecular modelling studies[14] of gagamine exhibits the carbonyl carbon of nicotinoyl group and C-20-OH to be close each other (distance: 3.68 Å). In a protic solvent system such as methanol, the carbonyl group may be attacked more easily by hydroxyl group of C-20-OH to form a seven-membered ring intermediate. Decomposition of this intermediate by ring opening provides the first migration product **1a**. The C-20-*O*-nicotinoyl group in **1a** further migrated to C-17 *via* a five-membered intermediate, affording the second migration product **1b**, which was easily converted to enol **1c** by elimination of nicotinic acid *via* a six-membered intermediate. Finally, the enol form was tautomerized to keto form to yield lineolon (**2**).[15]

In conclusion, gagamine (12-*O*-nicotinoyl-17-hydroxypregnenes) (**1**) can be converted into lineolon (12-hydroxy-17α-pregnenes) (**2**) *via* two successive acyl migrations, followed by elimination in a hot protic solvent system under solvolytic conditions. This kind of conversion must be a specific case of acyl migration.

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13. The mother liquid obtained from recrystallization of gagamine produced nicotinic acid, which was identified by the comparison of TLC and mixed melting point measurement (mp. 232-235 °C) with an authentic sample.
14. All calculations were run using the SYBYL 6.5 molecular modelling package (Tripos Associates, St. Louis, MO). Gasteiger-Hueckel charges: ε = 1, Energy: 38.47 kcal/mole, Distance: C52-O67 3.68, Energy increases only by 4.2 kcal/mole.
15. Lineolon (**2**): Colorless amorphous crystal, mp. 240-242 °C (242-247 °C).[7] IR (nujol): 3443 (OH), 1684 cm^{-1} (CO). EI-MS (70 eV) *m/z*: 364, 346 (M^+, base peak), 313, 180, 147, 120, 105, 97, 79, 43. HR-EI-MS *m/z*: 364.22442 (Caled for $C_{21}H_{33}O_5$: 364.22496). CD (c = 0.005, MeOH) $\Delta\varepsilon$ (nm): -1.20 (283). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.10 (1α-H), 1.17 (19-CH_3), 1.26 (18-CH_3), 1.47 (9-H), 1.53 (3-OH), 1.59 (2β-H), 1.61 (11α-H), 1.69 (8-OH), 1.71 (15β-H), 1.82 (15α-H), 1.84 (2α-H), 1.87 (11β-H), 1.91 (1β-H), 1.95 (16α-H), 2.05 (16β-H), 2.16 (7α-H), 2.22 (7β-H), 2.25 (21-CH_3), 2.32 (4β-H), 2.36 (4α-H), 3.09 (14-OH), 3.40 (17-H), 3.56 (3-H), 3.72 (12-H), 4.00 (12-OH), 5.35 (6-H). Coupling constants (Hz): J (1α,1β) = ~13.5, J (1α,2β) = 3.8, J (2α,3α) = ~5, J (2β,3α) = ~10.8, J (3α,4α) = ~5, J (3α,4β) = 10.8, J (3α,3-OH) = ~4.2, J (6,7α) = 2.2, J (6,7β) = 5.2, J (7α,7β) = 15.6, J (9,11α) = 3.6, J (9,11β) = 13.4, J (11α,11β) = 13.2, J (11α,12) = 4.2, J (11β,12) = 11.8, J (12,12-OH) = 3.0, J (14-OH, 15α) = 2.3, J (15α,15β) = 14.2, J (15α,16α) = 11.8, J (15α,16β) = 5.4, J (15β,16α) = 4.8, J (15β,16β) = 10.1, J (16α,16β) = 13.2, J (16α,17) = 11.1, J (16β,17) = 9.3. ^{13}C NMR ($CDCl_3$): δ 13.0 (18-C), 19.0 (19-C), 23.3 (11-C), 27.2 (15-C), 30.9 (2-C), 31.9 (21-C), 33.2 (16-C), 34.3 (7-C), 37.0 (10-C), 38.7 (1-C), 42.0 (4-C), 44.1 (9-C), 55.9 (13-C), 60.7 (17-C), 68.3 (12-C), 71.9 (3-C), 74.6 (8-C), 85.7 (14-C), 117.5 (6-C), 141.2 (5-C), 214.8 (20-C).