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CONSTITUENTS OF FERNS. 1. POLYSTHICOL, A 24-ETHYL-4,4-DIMETHYL-PHYTOSTEROL FROM *Polysthicum aculeatum* (L.) Roth.[1]

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Abstract: Occurrence of phytosterol 1a formed by an unprecedented alkylation sequence is reported.

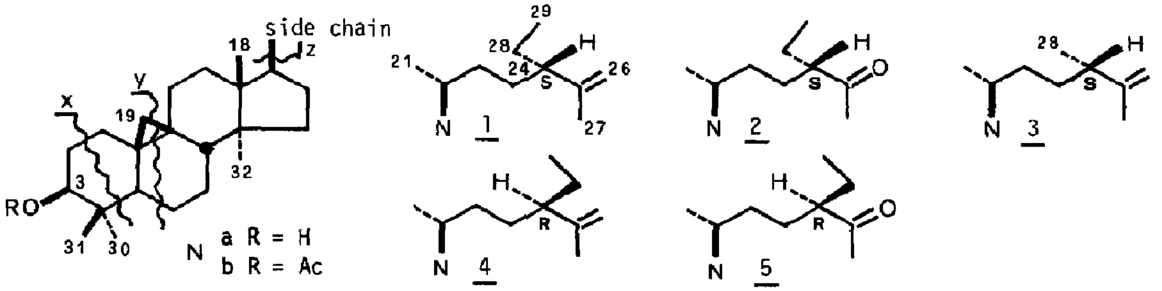
The second alkylation step in the biosynthetic sequence leading to phytosterols carrying an ethyl or ethylidene group at the 24-position of the side chain has been hitherto thought[2] to occur at the 4α-methyl sterol level or to an even later stage, since no 4,4-dimethyl sterol had yet been isolated which presented that structural feature. We have now found that leaves of the fern *Polysthicum aculeatum* (L.) Roth contain 24*S*-ethyl-9β,19-cyclo-5α-lanost-25-en-3β-ol ("polysthicol") 1a. Natural occurrence of this compound clearly suggests that reconsideration of the above aspect of phytosterol biosynthesis is needed.

The unsaponifiable fraction of the chloroform extract from leaves of the plant (collected in the Orto Botanico, Naples, in winter) was column (silica gel, 9:1 hexane-Et_2O) and PL (20% $AgNO_3$-silica gel, 9:1 $CHCl_3$-Me_2CO, 2 runs) chromatographed to give an alcohol mixture (IR: 3500-3600 cm^{-1}; ^1H-NMR: relevant signals at δ 3.2-3.5). Acetylation (Ac_2O/Py) followed by PLC (20% $AgNO_3$-silica gel, 1:1 C_6H_6-hexane, 4 runs) allowed pure acetate 1b (m.p. 139-41° (MeOH); $[\alpha]_D$ +51° ($CHCl_3$)) to be isolated. By hydrolysis (10% KOH/MeOH, 2 h, r.t.) alcohol 1a was recovered as a vitreous solid, $[\alpha]_D$ +42° ($CHCl_3$).

^1H-NMR spectrum (see Chart) of polysthicol showed no sgnificant differences with the spectrum of cyclolaudenol 3a,[3] the presence of a signal due to a primary methyl (δ 0.803t) in place of that due to the secondary (28)methyl of 3a excepted. Accordingly, in the ^{13}C-NMR spectrum a primary methyl resonance (δ 12.08) was displayed.[4] Structure 1a for the alcohol was also confirmed by MS (see Chart), as the molecular ion peak was seen at 454.41751 m/e (calc. for $C_{32}H_{54}O$: 454.41744), 14 mass unities more than 3a, and fragmentation pattern was in agreement with that reported[5] for 9,19-cyclolanostane phytosterols.

Normal absolute configuration was assigned to the tetracyclic skeleton by comparison of the sign and of the value of rotations of 1a and 1b to those of cyclolaudenol, cycloartanol, cycloartenol and 24-methylenecycloartanol and their acetates.[6] *S*-configuration to the C-24 chiral centre was established as follows. By $OsO_4/NaIO_4$ oxidation[7] polysthycol acetate 1b was converted into 2b which by treatment with MeONa/MeOH (2 h, r.t.) followed by re-acetylation (Ac_2O/Py) gave the 1:1 24-epimeric mixture 2b+5b. Wittig condensation ($\emptyset_3P^+CH_3Br^-$, n-BuLi, THF, 48 h, r.t.) gave back 1b besides its 24-epimer 4b. In the ^1H-NMR spectrum of this last mixture the 29-H_3 signal of the

CHART



1a ^{1}H-NMR, 270 MHz, $CDCl_3$, δ: 0.828d, 0.549d, J=4.15 Hz ($19-H_2$); 0.803t, J=7.35 Hz ($29-H_3$); 0.809s ($31-H_3$); 0.851d, J=6.25 Hz ($21-H_3$); 0.882s ($32-H_3$); 0.956s ($18-H_3$); 0.966s ($30-H_3$); 1.574s ($27-H_3$); 3.285dd, J=6.62 Hz, J=13.00 (3α-H); 4.648bd, 4.731bd, J=2.23 Hz ($26-H_2$). MS, m/e: 454.41751 (M^+), 439 ($M-CH_3$), 436 ($M-H_2O$), 421 ($M-CH_3-H_2O$), 393 ($M-H_2O-C_3H_7$), 367 (M-x-H), 315 (M-z), 314 (M-y-H), 297 ($M-z-H_2O$), 203, 175 (M-y-H-z), 95.

1b ^{1}H-NMR, 270 MHz, $CDCl_3$, δ: 0.338d, 0.569d, J=4.40 Hz ($19-H_2$); 0.803t (J=7.35 Hz ($29-H_3$); 0.846s ($31-H_3$); 0.849d, J=6.25 Hz ($21-H_3$); 0.887s ($32-H_3$); 0.887s ($30-H_3$); 0.952s ($18-H_3$); 1.574s ($27-H_3$); 2.055s (-OAc); 4.567dd, J=5.22 Hz, J=11.00 Hz (3α-H); 4.648bd, 4.734bd, J=2.2 Hz ($26-H_2$). MS, m/e: 496 (M^+), 481 ($M-CH_3$), 436 (M-AcOH), 421 ($M-CH_3-AcOH$), 393 ($M-AcOH-C_3H_7$), 384 ($M-C_8H_{16}$), 367 (M-x-H), 357 (M-z), 314 (M-y-H), 297 (M-z-AcOH), 203, 175 (M-y-H-z), 95.

2b ^{1}H-NMR, 270 MHz, $CDCl_3$, δ: 0.335d, 0.573d, J=4.23 Hz ($19-H_2$); 0.846s ($31-H_3$); 0.865d, J=6.62 Hz ($21-H_3$); 0.869t, J=7.35 Hz ($29-H_3$); 0.885s ($32-H_3$); 0.885s ($30-H_3$); 0.951s ($18-H_3$); 2.052s (-OAc); 2.108s ($27-H_3$); 2.323m, $W_{1/2}$=22 Hz (24-H); 4.565dd, J=4.78 Hz, J=11.03 Hz (3α-H). MS, m/e: 498 (M^+), 483 ($M-CH_3$), 438 (M-AcOH), 423 ($M-CH_3-AcOH$), 395 ($M-AcOH-C_3H_7$), 369 (M-x-H), 357 (M-z), 316 (M-y-H), 297 (M-z-AcOH), 203, 175 (M-y-H-z), 95.

new epimer appears at an upper field (δ 0.798t) and the $21-H_3$ signal at a lower field (δ 0.858d) than the corresponding signals of the natural epimer (δ 0.803t and δ 0.849d, resp.). This clearly indicates[8] the 24*S* configuration for natural polysthicol 1a.[9]

REFERENCES AND NOTES

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9. Accordingly,[8] in the spectrum of the mixture 2b+5b the $29-H_3$ signal of the new epimer (5b) was found at an higher field (δ 0.858t) than that of 2b (δ 0.869t).

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