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ERIANOL, A 4α-METHYLSTEROL FROM THE ORCHID *ERIA CONVALLARIOIDES*

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Key Word **Index**—*Eria convallarioides*; Orchidaceae; sterol; erianol; nudol; erianthridin; sitosterol; 4α,24,24-trimethylcholesta-7,25-dien-3β-ol.

Abstract-From the orchid ***Eria convallarioides*** nudol, erianthridin, sitosterol and an uncharacterized fatty alcohol, along with a new steroidal compound, designated as erianol, which constitutes a modified intermediate in the biogenetic transformation of lanosterol to the steroidal system were isolated. The structure of erianol was established as 4α,24,24-trimethylcholesta-7,25-dien-3β-ol from spectral and chemical evidence.

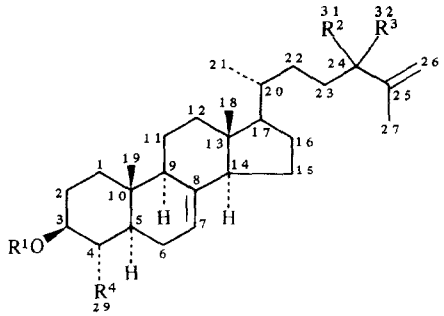
INTRODUCTION

From a series of Indian orchids we reported [1–20] the isolation of a number of compounds which represent several structural types such as bibenzyls [1, 2], phenanthrenes [3–7], phenanthropyrans [8], 9,10-dihydrophenanthrenes [9, 10], 9,10-dihydrophenanthropyrans [11–15, 173, and pyrones [11-13, 16], triterpenoids [18, 19] and steroids [20]. Our continued search for new phytochemicals from the same source has resulted in the isolation of another new steroidal compound, designated as erianol, from the methanolic extract of the orchid ***Eria convallarioides,*** besides nudol (**2a**) [3, 21], erianthridin (2b) [10], sitosterol and an uncharacterized fatty alcohol. The structure of erianol was established as la from the following spectral and chemical evidence.

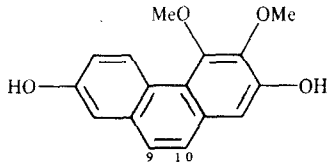
RESULTS AND DISCUSSION

Erianol, $C_{30}H_{50}O$ (M^+ at m/z 426), mp 168°, $[\alpha]_D$ + 3.5° ($CHCl_3$), showed in its IR spectrum bands for hydroxyl group (ν_{max} 3420 cm^{-1}), a trisubstituted double bond (ν_{max} 1653 cm^{-1}) and a terminal methylene function (ν_{max} 1630 and 888 cm-'). The presence of a hydroxyl group in erianol was confirmed by the formation of an acetyl derivative, $C_{32}H_{52}O_2$ (M^+ at m/z 468), mp 102". The ^1H NMR spectrum of erianol showed an ill-resolved two-proton doublet at δ 4.69 and a three-proton singlet at 1.69, which together indicated the presence of the terminal methylene group to be associated with the system -C-C (Me)=CH_2 as in otochilone (3) [20] and cyclootochilone (4) [20]. The spectrum also exhibited signals for an additional olefinic proton associated with a trisubstituted double bond (6 5.17, ill-resolved multiplet), six methyl groups linked to sp^3 carbon atoms (1.07, 6H, *s*; 0.83 and 0.53, each 3H, *s*; 0.92 and 0.99, each 3H, *d*, *J* = 7 Hz) and a hydroxy methine proton (3.12, doublet of triplet, J_1 = 6.32 Hz and J_2 = 2.3 Hz). The last signal was shifted to δ 4.38 (doublet of triplet, J_1 = 10 Hz and J_2 = 3.5 Hz) in the spectrum of erianol acetate. The chemical shifts and the splitting patterns of the above methine protons in the two compounds suggested the equatorial disposition of the hydroxyl group in erianol.

Treatment of erianol acetate with $Hg(OAc)_2$ in glacial acetic acid [22] afforded a compound, $C_{32}H_{50}O_2$ (M^+ at *m/z* 464), mp 158°, which showed UV absorption at λ_{max} 241 nm (log ε 4.21), characteristic of a 7,9-heteroannular diene chromophore in a lanostane skeleton [22, 23], rather than that in the tirrucalane (antipodal at C-13, C-14 and C- 17) or euphorbane (antipodal at C- 13 and C-14) system. A 7,9-heteroannular diene of the latter two types of compounds would have shown three UV absorption maxima at ~ 223, ~ 247 and ~ 258 nm [22, 23]. The structure of the heteroannular diene derived from erianol acetate was shown to have a lanostane skeletal structure lc from its various spectral data. The stereochemistry of erianol at C-13, C-14 and C-17 is, therefore, identical with those at the corresponding carbon atoms of lanostane. The trisubstituted double bond in erianol was placed at the A'-position on the basis of the relatively upfield position (6 5.17) of the olefinic proton. An olefinic proton associated with a $\Delta^{9(11)}$-double bond was found to resonate at a slightly lower field (6 5.23) [24]. The tetracyclic formulation la for erianol bearing a A'-double bond and a side chain identical with those of otochilone (3) [20] and cyclootochilone (4) [20] was also consistent with its mass spectral fragmentation. The mass spectrum of erianol showed significant peaks at *m/z* 426 $[M]^+$, 286 [M-side chain $-H]^+$, 285 [M-side chain $-2H]^+$, 269, 267, 245 (ion-fragment derived from M^+ by the cleavage of 13, 17 and 14, 15 bonds and loss of a H), 229 and 83 (attributed to the highly stabilised ion-fragment $\dot{C}(Me)_2C(Me)=CH_2$). While the peak at *m/z* 83, which is also discernible in the mass spectra of both otochilone (3) [20] and cyclootochilone (4) [20], further corroborates the identical nature of the side chain in the three compounds, other peaks, particularly that at *m/z* 245, are characteristic [25] of a A'-bond in erianol (**la**). An alternative formulation with a $\Delta^{9(11)}$-double bond would have shown a completely different [26] mass spectral fragmentation.

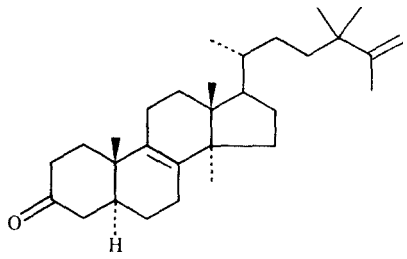


1a R^1 = H, R^2 = R^3 = R^4 = Me
1b R^1 = Ac, R^2 = R^3 = R^4 = Me
1c R^1 = Ac, R^2 = R^3 = R^4 = Me; 9,11-dehydro
1d R^1 = R^2 = R^4 = H, R^3 = Me; 22,23-dehydro



2a

2b 9.10-dihydro



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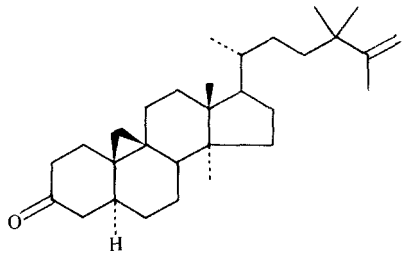

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More compelling evidence for the structure **1a** was provided by its ^{13}C NMR spectral data (Table 1). The degree of protonation of each carbon atom was determined by DEPT experiments and the assignments of the carbon chemical shifts were made by comparison with the δ_c values of the corresponding carbon atoms in structurally similar compounds [27, 20]. Thus, C-20, C-21, C-22, C-23, C-24, C-25, C-26, C-27, C-31 and C-32 of erianol constituting its side chain appeared essentially at the same positions as the corresponding carbon atoms of otochilone (3) [20] and cyclootochilone (4) [20] confirming the assigned structure of its side chain. The absence of a methyl group at C- 14 of erianol was evident from the observed changes in the δ_c values of its C-12, C-13, C-14, C-15, C-16, C-17 and C-18 compared to the corresponding carbon atoms of otochilone and cyclootochilone and other related compounds possessing a methyl group at C-14. This was further confirmed by the fact that the above carbon atoms of erianol appeared almost at the same positions as the corresponding carbon atoms of **1d** [27] and similar other compounds having no methyl group at C-14 [27]. Furthermore, C-7 and C-8 of erianoi also appeared essentially at the same positions as the corresponding carbon atoms of **1d**. A methyl group at C-14 in erianol would have caused a deshielding of C-8 (by an additional p-effect) and a shielding of C-7 (by an additional y-effect). The presence of a single methyl group at C-4 of erianol and its equatorial disposition is consistent with the observed upfield shift of its C-3 and C-5 (removal of a p-axial methyl effect) and the downfield shift of its C-2 and C-6 (removal of a y-effect) compared to the corresponding carbon atoms of structurally similar compounds bearing a *gem*-dimethyl group at C-4 [27]. The relatively greater shielding of C-5 of erianol may be attributed to an additional homoallylic shielding caused by the Δ^7-double bond which also exerts a similar effect

Table 1. ^{13}C NMR chemical shifts of erianol (la)*

C	δ	C	δ_1
1	36.99	16	27.91
2	30.96"	17	55.85
3	76.22	18	11.76
4	40.25	19	14.13[b]
5	46.65	20	36.64
6	26.64	21	18.99
7	117.47	22	30.41"
8	139.14	23	36.99
9	49.64	24	38.67
10	34.83	25	152.37
11	21.35	26	109.28
12	39.33	27	19.35
13	43.33	29	15.14[b]
14	54.94	31	27.52'
15	22.9	32	27.18'

*Values are in ppm downfield from TMS: $\delta_{(TMS)} = \delta_{(CDCl_3)} + 76.9$ ppm.
[a–c] Values are interchangeable.

on its C-10 compensating the deshielding effect caused by the removal of a methyl group at C-4. The equatorial nature of the methyl group at C-4 in erianol is also in accord with biogenetic consideration and with the splitting patterns of H-3 of erianol and its acetate.

The structure of erianol is thus established as **4α,24,24-trimethylcholesta-7,25-dien-3β-ol (1a)** and it represents a biogenetic intermediate in the transformation of lanosterol to a steroid system [28–30], which has been further modified in the side chain by incorporating two methyl groups at C-24 through a process similar to that envisaged in the case of otochilone (3) [20] and cyclootochilone (4) [20].

EXPERIMENTAL

Mps: uncorr. IR spectra were measured in KBr discs and UV spectrum in 95% aldehyde-free EtOH. ^{1}H NMR spectra were recorded at 250 and 300 MHz in $CDCl_3$ soln. using TMS as int. standard. ^{13}C NMR spectra were measured at 62.5 MHz using the same solvent and int. standard. Chemical shifts were measured in δ ppm and for ^{13}C NMR $\delta_{TMS} = \delta_{CDCl_3} + 76.9$ ppm. MS were recorded at 70 eV. Silica gel (100–200 mesh) were used for CC and silica gel G for TLC. All analytical samples were routinely dried over P_2O_5 for 24 hr *in vacuo* and were tested for purity by TLC and MS. Dry Na_2SO_4 was used for drying organic solvents and petrol used had bp 60–80°. Known compounds were identified by direct comparison (mp, mmp and IR spectra) with respective authentic samples.

***Isolation of erianol* (1a), *nudol* (2a) and** erianthridin **(2b).** Air-dried powdered whole plant of *E. convallarioides* (2 kg) was soaked in MeOH (5 1) for 3 weeks. The MeOH extract was concd under red. pres. to ~ 100 ml, diluted with H_2O (750 ml) and exhaustively extracted with Et_2O. The Et_2O layer was then extracted with 2 M aq. NaOH soln. to remove the phenolic and acidic constituents. The resultant Et_2O extract containing the neutral constituents was then washed with H_2O, dried and the solvent removed. The residue was chromatographed. The petrol-EtOAc (100: 1) eluate gave an uncharacterized fatty alcohol, mp 72". Washing the column with petrol-EtOAc (50: 1) as eluent afforded **1a** (0.1 g), crystallized from petrol-EtOAc, mp 168°, $[\alpha]_D + 3.5°$ ($CHCl_3$). (Found: C, 84.46; H, 11.75; $C_{30}H_{50}O$ requires: C, 84.50; H, 11.73%). IR ν_{max} cm^{-1}: 3420 (OH), 1653 (trisubstituted double bond), 1630 and 888 (C=CH_2); MS m/z (rel. int.); 426 [M]$^+$, (11), 412 (10), 411 (12), 408 (3), 393 (5), 313 (6), 286 (13), 285 (57), 271 (10), 269 (25), 268 (1 1), 267 (18), 260 (8), 255 (6), 245 (13), 243 (9), 241 (10), 229 (14), 226 (19), 213 (10), 187 (ll), 175 (12), 173 (17), 161 (27), 159 (23), 157 (11), 149 (15), 147 (34), 145 (27), 137 (14), 135 (28), 133 (30), 131 (15), 124 (30), 123 (30), 121 (40), 120 (15), 119 (35), 109 (40), 108 (19), 107 (40), 105 (40), 97 (23), 95 (63), 93 (40), 91 (23), 85 (23), 84 (29), 83 (59), 81 (56), 79 (24), 71 (18), 69 (70), 67 (28), 57 (49), 55 (100), 43 (59) and 41 (40).

Compound **1a** was acetylated with Ac_2O and pyridine in the usual manner to give **1b,** crystallized from petrol-EtOAc mixture, mp 102". (Found: C, 82.10; H, 11.03; $C_{32}H_{52}O_2$ requires: C, 82.05; H, 11.11%). IR ν_{max} cm^{-1}: 1710 and 1250 OAc); ^{1}H NMR: 6 5.15 (1H, *m*, H-7). 4.66 (2H, apparent *d*, H_2-26), 4.38 (1H, *dt*, J_1 = 10 Hz, J_2 = 3.5 Hz, H-3), 2.11 (3H, s, –OCO Me), 1.66 (3H, *s*, Me–C=C), 1.04 (6H, *s*, 2 × -C-Me), 0.89 (3H, *d*, J = 6 Hz; -CH-Me), 0.83 (3H, *d*, J = 7 Hz, CH-Me), 0.81 and 0.5 (each 3H, s, 2 × –C–Me). The petrol-EtOAc (30: 1) eluate from the chromatography of the neutral constituent afforded sitosterol, mp 137".

The aq. alkaline soln obtained in the fraction of the total methanolic extract was acidified in the cold with conc HCI and the liberated solid extracted with Et_2O, washed with H_2O, dried and the solvent removed. The residue was chromatographed and the early fractions eluted with petrol-EtOAc (10: 1) gave mainly **2a** mixed with small amounts of **2b.** The mixture on repeated chromatography finally afforded pure **2a** (0.05 g), crystallized from petrol-EtOAc, mp 185". The later fraction of the same eluate gave a mixture of **2a** and mainly **2b**. Repeated chromatography of this mixture finally gave pure **2b** (0.03 g), crystallized from petrol-EtOAc, mp 145".

***Formation of the heteroannular diene* (1c).** To a soln of **1b (0.03 g)** in glacial HOAc (6 ml) was added 0.07 g $Hg(OAc)_2$ in 3 ml glacial HOAc and the mixture stirred at room temp. for 24 hr. The liberated $Hg_2(OAc)_2$ was filtered off and to the filtrate was added, in portions, $NaBH_4$ (0.1 g) under continuous stirring. The black ppt. of Hg was then filtered off and the filtrate evapd under vacuum. The residue was treated with H_2O (10 ml) and extracted with Et_2O. The Et_2O extract was washed with H_2O, dried and the solvent removed. The residue was chromatographed and the petrol-EtOAc (50: 1) eluate gave **1c** (0.02 g), crystallized from petrol-EtOAc, mp 158". IR ν_{max} cm^{-1}: 1740 and 1240 (OAc), 1660 and 1640 (trisubstituted double bond), 1625 and 888 (terminal double bond); ^{1}H NMR: δ 5.38 and 5.63 (each 1H, *m*, H-7 and H-l1), 4.66 (2H, *m*, H_2-26), 4.39 (1H, *m*, H-3), 2.08 (3H, s, –OAc), 1.66 (3H, *s*, Me–C=C) and 0.5-1.08 (6 x-C-Me).

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