EXPERIMENTAL

The air dried aerial parts of *M. zeylanica* (400 g, collected in Sri Lanka, voucher No. NKBA 34) was extracted with Et_2O–MeOH–petrol, 1:1:1, and worked-up as reported previously [9]. By CC (SiO_2) and TLC (SiO_2, PF 254) 40 mg β-farnesene, 20 mg squalene, 25 mg dammadienyl acetate, 20 mg caryophyllen-1,10-epoxide, 20 mg **1** and 5 mg 5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone were obtained and identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic material. The polar CC fraction (Et_2O–MeOH, 9:1) gave by HPLC (RP 8, MeOH–H_2O, 7:3, *ca* 100 bar) 2 mg **3** (R_t 3.5 min) and 10 mg **2** (R_t 4.0 min).

10,11-*Dihydromicroglossic acid* (**2**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500–2700, 1720 (CO_2H), 1740 (CO_2R); MS m/z (rel. int.): 364.225, $[M]^+$ (2) (calc. for $C_{21}H_{32}O_5$: 364.225), 346 $[M - H_2O]^-$ (14), 332 $[M - MeOH]^+$ (5), 324 $[346 - MeOH]^+$ (7), 108 (51), 95 (74), 94 (72), 83 (98), 82 (98), 81 (68), 69 (100).

Microglossic acid (**3**). Colourless oil; MS m/z (rel. int.): 362.209 $[M]^+$ (6) (calc. for $C_{21}H_{30}O_5$: 362.209), 330 $[M - MeOH]^+$ (5), 284 $[330 - HCO_2H]^+$ (10), 256 $[284 - CO]^+$ (12), 121 (60), 69 (100).

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DITERPENOIDS FROM *VELLOZIA FLAVICANS*

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Key Word Index—*Vellozia flavicans*; Velloziaceae; diterpenes; cleistanthanes.

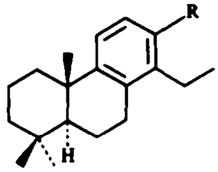
Abstract—The isolation of three new cleistanthane diterpenes from *Vellozia flavicans* is described and their absolute configurations have been determined by chemical correlation.

INTRODUCTION

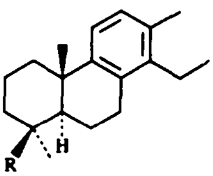
In a previous communication, we described the isolation of veadeirol (**1**) and veadeiroic acid (**2**) from the roots, stems and leaf sheaths of *Vellozia flavicans* [1], and we further determined their absolute configurations [2]. We report now the isolation of three new oxygenated cleistanthane diterpenes from the same plant. These have been identified as (4*R*, 5*S*, 10*S*)-cleistantha-8,11,13-trien-19-ol (**3**), (4*R*, 5*S*, 10*S*)-cleistantha-8,11,13-trien-19-oic acid (**4**) and (4*R*, 5*S*, 10*S*)-cleistantha-8,11,13-trien-19-al (**7**).

RESULTS AND DISCUSSION

Fractionation of the hexane extract of *V. flavicans* afforded, in addition to the diterpenes **1** and **2**, an inseparable mixture of alcohols **1** and **3**, and acid **4** invariably contaminated by **2**. Hydrogenolysis of the alcohol mixture yielded unreacted **3** and hydrocarbon **5** which were easily separated by silica gel column chromatography affording pure **3** as a minor constituent (mp 110–112°; $[\alpha]_D + 33°$). Acid **4** could only be purified as its methyl ester derivative **6** (mp 92–95°; $[\alpha]_D + 92°$).



1 R = CH_2OH
2 R = COOH
5 R = Me
8 R = CHO



3 R = CH_2OH
4 R = COOH
6 R = COOMe
7 R = CHO
9 R = CH_2OTs

The molecular formula of **3** ($C_{20}H_{30}O$) was determined by a combination of low resolution mass spectrometry and ^{13}C NMR spectroscopy, whereas that of **6** ($C_{21}H_{30}O_2$) came from low resolution mass spectrometry only. Their ^1H NMR spectra contained resonances assigned to four methyl groups, two of which were on quaternary carbons, one on an aromatic nucleus and the fourth was part of an ethyl moiety. These data, and the presence of a 1,2,3,4-tetra-substituted aromatic ring (IR, ^1H and ^{13}C NMR spectra), suggested that these substances were tricyclic diterpenes, probably of the cleistanthane class.

The oxygen-containing functional group of **3** was shown to be a primary hydroxyl group by its IR spectrum (3330 cm^{-1}) and by the presence of two doublets (AB system) at $\delta 3.55$ ($J = 11$ Hz) and 3.87 ($J = 11$ Hz) in the ^1H NMR spectrum. The intense ion observed in the mass spectrum of **3** at m/z 271 $[M - 15]^+$ (53%) corresponded to the loss of the angular methyl group at C-10. This fragmentation, common in the abietatriene series [3], suggested the presence of the hydroxymethyl group at C-4. Localization of the hydroxyl group at the C-19 axial position was deduced from the chemical shifts of C-18 and C-5 ($\delta 26.9$ and 51.0 respectively [4]), which were found to be in agreement with the proposed stereochemistry [4]. Compound **3** was thus identified as cleistantha-8,11,13-trien-19-ol.

The second diterpene of the cleistanthane type, acid **4**, was purified and analysed as its methyl ester derivative **6** (IR: 1715 cm^{-1}). The ^1H NMR spectrum of **6** was similar to that of **3** except for the absence of the hydroxymethyl AB system and for the presence of a 3H singlet at $\delta 3.66$ due to the methoxyl of the ester group. Lithium aluminium hydride reduction of **6** yielded **3** identical in all aspects with the diterpene isolated from the plant.

The presence of both **3** and **4** in *V. flavicans* suggested the existence of the corresponding aldehyde (**7**). This compound was indeed detected by ^1H NMR spectroscopy but it could never be obtained pure, being always contaminated by veadeiral (**8**) [1]. Characteristic ^1H NMR signals of **7** were a 1 H singlet at $\delta 9.80$ (CHO), two 1 H doublets ($J = 8.5$ Hz) at $\delta 6.93$ and 7.03 (ArH) and a 3H singlet at $\delta 2.29$ (ArMe). Sodium borohydride reduction of mixture **7** and **8** afforded an inseparable mixture of alcohols unambiguously identified as **3** and veadeirol (**1**) by ^1H NMR spectroscopy. Final proof for structure **7** came by oxidation of pure alcohol **3**. Oxidation in Swern conditions [5] afforded a complex mixture, probably of rearranged compounds, in which aldehyde **7** was a minor component. On the contrary, Jones oxidation of **3** yielded mainly acid **4** and traces of aldehyde **7** identical with natural **7** by ^1H NMR spectroscopy. This reaction allowed the assignment of the structure of **7** by chemical correlation with **3**.

Finally, the absolute configurations and the cleistanthane skeleton of diterpenes **3**, **4** and **7** were deduced in the following way. Tosylation of **3** to **9** and subsequent lithium aluminium hydride reduction of the latter afforded hydrocarbon **5**, identical by spectral data, inclusive of the optical rotation, to an authentic sample of known absolute configuration [2].

Diterpenes **3**, **4** and **7** are the first known cleistanthanes from Velloziaceae with an oxygen function at C-19.

EXPERIMENTAL

All the equipment used for this study has been described earlier [2]. When reaction mixtures were worked up in the usual way they were diluted with H_2O, extracted with $CHCl_3$, dried over dry $MgSO_4$, filtered and evaporated to dryness under red. pres.

Isolation of diterpenes **1**–**5**. *Vellozia flavicans* (3.5 kg dry wt) was pulverized in a hammer mill and extracted exhaustively with hexane at room temp. Evaporation under red. pres. of the solvent afforded a brownish gummy residue (90 g). Extensive silica gel CC (elution with a gradient of EtOAc in hexane from 0 to 50%) afforded a mixture of aldehydes **7** and **8**, veadeirol (**1**, 2 g) [1], a mixture of **1** and **3** (223 mg), veadeiroic acid (**2**, 600 mg) [1] and a mixture of acids **2** and **4** (63 mg). Veadeirol (**1**, mp 138–139°; $[\alpha]_D^{CHCl_3} + 35.0°$, $c = 2.0$) and veadeiroic acid (**2**, mp 226–227°; $[\alpha]_D^{MeOH} + 44.3°$, $c = 0.31$) were identified by comparison with authentic samples [1].

Isolation of pure alcohol **3**. A mixture of **1** and **3** (115 mg, relative proportions 1:2) in AcOH (10 ml) was treated, at room temp., under an atmosphere of H_2 (62 p.s.i.) in the presence of catalytic amount of 10% Pd–C. After 3 hr, the catalyst was removed by filtering. The reaction mixture was worked up in the usual way to furnish a mixture of hydrocarbon **5** and unreacted **3**. Silica gel CC (hexane–EtOAc, 85:15) afforded pure **5** (35 mg) identified by comparison with an authentic sample [2] and pure alcohol **3** (73 mg).

Compound **3**: mp 110–112°; $[\alpha]_D^{25} + 33°$, $c = 0.2$ in $CHCl_3$; IR ν_{max}^{film} cm^{-1}: 3320 (OH), 1025 and 815 (1,2,3,4-tetrasubstituted aromatic ring); MS m/z (rel. int.): 286 (M^+, $C_{20}H_{30}O$, 22) 271 (51), 253 (33), 213 (9), 199 (7), 185 (22), 183 (16), 173 (28), 171 (11) and 159 (100); ^1H NMR (100 MHz, $CDCl_3$): 1.06 (3H, *t*, $J = 6.5$ Hz, Me-16), 1.06 (3H, *s*, Me-20), 1.21 (3H, *s*, Me-18), 2.29 (3H, *s*, Me-17), 2.63 (2H, *q*, $J = 6.5$ Hz, C-15 H_2), 3.55 (1H, *d*, $J = 11$ Hz, H-19) 3.87 (1H, *d*, $J = 11$ Hz, H′-19) and centered at 7.00 (2H, AB system, $J = 8$ Hz, H-11 and H-12); ^{13}C NMR (25.2 MHz, $CDCl_3$): 148.92 (*s*, C-9), 141.23 (*s*, C-14), 133.69 (*s*, C-8*), 133.39 (*s*, C-13*), 128.81 (*d*, C-12), 122.95 (*d*, C-11), 65.70 (*t*, C-19) 50.98 (*d*, C-5), 39.67 (*t*, C-1), 38.89 (*s*, C-4**), 38.11 (*s*, C-10**), 35.37 (*t*, C-3), 28.40 (*t*, C-7), 26.92 (*q*, C-18), 26.07 (*q*, C-20), 22.30 (*t*, C-15), 19.50 (2*t*, C-2 and C-6), 19.26 (*q*, C-17) and 13.06 (*q*, C-16).

Preparation of methyl ester **6**. A crude mixture of acids **2** and **4** (57 mg) in dry Et_2O (5 ml) was treated overnight, at room temp., with a saturated Et_2O soln of freshly prepared CH_2N_2. The excess of reagent was destroyed by addition of a few drops AcOH, and the solvents were evaporated under red. pres. to furnish a mixture of the corresponding methyl esters from the which compound **6** was isolated by silica gel CC (hexane–EtOAc from 100:0 to 80:20). Crystallization from hexane–EtOAc yielded pure **6**: mp 92–95°; $[\alpha]^{25} + 92°$ (589), $+ 96°$ (578), $+ 109°$ (546) and $+ 186°$ (436 nm); IR ν_{max}^{KBr} cm^{-1}: no OH group, 2935, 1715, 1450, 1355, 1315, 1217, 1202, 1175, 1130, 1075, 1015, 970, 966, 810 and 762; MS m/z (rel. int.): 314 (M^+, $C_{21}H_{30}O_2$, 14), 299 (37), 285

(2), 267 (4), 255 (3), 239 (100), 225 (4), 211 (5), 209 (3), 197 (4), 195 (3), 191 (2), 183 (15), 181 (4), 173 (10), 159 (18), 157 (12), 155 (9), 142 (8), 128 (8), 43 (6), 41 (6), and 34 (75); ^{1}H NMR (100 MHz, $CDCl_3$): 1.06 (3H, *s*, Me-20), 1.12 (3H, *t*, J = 7 Hz, Me-16), 1.28 (3H, *s*, Me-18), 2.29 (3H, *s*, Me-17), 2.63 (2H, *q*, J = 7 Hz, C-15 H_2), 3.66, (3H, *s*, OMe), 6.93 and 7.05 (1H each, *d*, J = 8 Hz, H-11 and H-12).

Lithium aluminium hydride reduction of **6**. Ester **6** (23 mg), dissolved in dry THF (5 ml), was refluxed during 3 hr in the presence of $LiAlH_4$ (47 mg). The excess reagent was then destroyed by successive addition of EtOAc, EtOH, H_2O and 2N H_2SO_4 (2 ml). The reaction mixture was worked up in the usual way to furnish, after purification by preparative TLC on silica gel, pure alcohol **3** (20 mg) identical by $[\alpha]_D$, IR, MS and ^{1}H NMR to natural **3**.

Reduction of mixture **7** *and* **8**. A mixture of **7** and **8** (62 mg, relative proportions 1:1) in MeOH (5 ml) was treated at room temp., during 1.5 hr, with an excess of $NaBH_4$. The excess of reagent was then destroyed by addition of Me_2CO and the reaction mixture was worked up in the usual way to afford a crude mixture of alcohols **1** and **3**. Purification by preparative TLC on silica gel yielded the mixture of **1** and **3** spectroscopically identical to the natural mixture.

Jones oxidation of **3**. Compound **3** (26 mg), in Me_2CO (2 ml), was treated at room temp. with Jones reagent until an orange colour remained. The excess of reagent was then destroyed by addition of *i*-PrOH and the reaction mixture was worked up in the usual way to afford crude **7** which was purified by preparative TLC (hexane–EtOAc 90:10). This afforded slightly contaminated **7** (6 mg) along with acid **4** (17 mg).

Compound **7**: an oil $[\alpha]_D^{25} + 66.5°$, $c = 1.94$ in $CHCl_3$; IR ν_{max}^{film} cm^{-1}: 2940, 2710, 1725, 1460, 1380, 1125 and 815 (1,2,3,4-tetrasubstituted aromatic ring); ^{1}H NMR (100 MHz, $CDCl_3$): 1.10 (6H, *s*, Me-18 and Me-20), 1.11 (3H, *t*, J = 7 Hz, Me-16), 2.29 (3H, *s*, Me-17), 2.63 (2H, *q*, J = 7 Hz, C-15 H_2), 6.93 and 7.03 (1H each, *d*, J = 8.5 Hz, H-11 and H-12) and 9.80 (1H, *s*, H-19).

Tosylation of **3**. Alcohol **3** (18 mg), in dry pyridine (1 ml), was treated with TsCl (30 mg) at room temp. during 1 day. The reaction mixture was worked up in the usual way, and the crude tosylate **9** was purified by preparative silica gel TLC (hexane–EtOAc, 90:10) to afford pure **9** (15 mg) characterized by its ^{1}H NMR (100 MHz, $CDCl_3$) spectrum: 1.04 and 1.08 (3H each, *s*, Me-18 and Me-20), 1.10 (3H, *t*, J = 7 Hz, Me-16), 2.30 (3H, *s*, Me-17), 2.48 (3H, *s*, Me(OTs)), 2.60 (2H, *q*, J = 7 Hz, C-15 H_2), 3.92 (1H, *d*, J = 9 Hz, H-19), 4.26, (1H, *d*, J = 9 Hz, H′-19), 6.96 (2H, *s*, H-11 and H-12), 7.34 (2H, *d*, J = 8 Hz, Ts-H) and 7.80 (2H, *d*, J = 8 Hz, Ts-H).

Lithium aluminium hydride reduction of **9**. Tosylate **9** (15 mg), in dry THF (2 ml), was refluxed with an excess of $LiAlH_4$ for 12 hr. After cooling, the reaction mixture was treated as above. Purification of crude extract by preparative silica gel TLC hexane–EtOAc, 95:5) yielded pure **5** (9 mg) which was identical by $[\alpha]_D$, IR, UV, MS and ^{1}H NMR with an authentic sample [2].

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