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DITERPENES FROM *VELLOZIA NANUZAE*

ANGELO C. PINTO, ROSALY S. DA SILVA and LIGIA M. M. VALENTE

Instituto de Química, Universidade Federal do Rio de Janeiro, Centro de Tecnologia, Bloco A, Cidade Universitária, Ilha do Fundão-21910, Rio de Janeiro, R.J., Brasil

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Key Word Index—*Vellozia nanuzae*; Velloziaceae; pimarane-type diterpenes; structure elucidation.

Abstract—Two new diterpenes, nanuzone and 11β-hydroxy-nanuzone, have been isolated from *Vellozia nanuzae*. Their structures were elucidated by spectroscopic methods and confirmed by partial synthesis.

INTRODUCTION

As part of a continuing chemical investigation on Brazilian Velloziaceae, we have examined a sample of *Vellozia nanuzae* L. B. Smith & Ayensu collected in Serra do Cipó, Minas Gerais, Brazil. From the hexane, ethyl acetate and ethanol extracts of roots, stem and leaf sheaths of this plant we have isolated β-sitosterol and stigmasterol as a mixture, oleic acid, palmitic acid, caffeic acid, 20-hydroxy-lupan-3-one (**1**) [1], 12β-hydroxy-8(9),15-isopimaradien-7-one (**2**) [2, 3], 11β-hydroxy-20-nor-8(9),15-isopimaradien-7-one (**3**) [4], 7,16-epoxy-20-nor-5,7,9,11,13-cleistanthapentaen-3-one (**4**), 7,16-epoxy-20-nor-1,5,7,9,11,13-cleistanthahexaen-3-one (**5**) [5], (5*R*,7*S*,10*S*)-7α,16;7β,20-diepoxy-1,8,11,13-cleistanthatetraen-3-one (**6**), and (5*R*,7*S*,10*S*)-7α,16;7β,20-diepoxy-8,11,13-cleistanthatrien-3-one (**7**) [6, 7] all of which have already been isolated from other Brazilian Velloziaceae, and two new pimarane diterpenes nanuzone (**8**) and 11β-hydroxy-nanuzone (**9**) each of which contains a 15,16-epoxy moiety in place of the more common 13-vinyl function. This paper describes the structure elucidation of nanuzone and 11β-hydroxy-nanuzone.

RESULTS AND DISCUSSION

Nanuzone (**8**) is a colourless crystalline compound, mp 133–135° $[\alpha]=+50$ ($CHCl_3$; *c* 0.78), molecular formula $C_{20}H_{30}O_2$ (HRMS). Its UV spectrum displayed one maximum absorption at 251 nm ($\log \varepsilon = 4.10$) and showed IR bands at 1660 and 1620 cm^{-1}, characteristic of an α,β-unsaturated ketone. No bands of hydroxyl groups were present in the IR spectrum. The 100 MHz ^1H NMR spectrum ($CDCl_3$) showed four three-proton singlets at δ 0.86, 0.89, 0.93 and 1.10, indicating four methyl groups linked to quaternary carbons, and signals of three protons in a complex pattern (δ 2.4–2.8). No signals for olefinic protons were observed. All these data and biogenetic considerations are consistent with a pimarane-type diterpene with a keto group conjugated with a 8(9)-double bond, and a 15ξ,16-epoxide ring in place of the more common vinyl group at C-13. The presence of the epoxy moiety was confirmed by the ^{13}C NMR data (Table 1). The keto group must be placed at C-7, rather than C-11, because of its shielding effects on C-14 (*s* 31.3) [8]. Furthermore, oxy functions at C-11 should cause a downfield shift of the C-10 methyl signal larger than that observed for the Me-20 (^1H NMR) [3].

The configuration at C-13 was determined by the chemical shift of C-17 (δ 22.7) which indicates it to be in an equatorial position [8]. The A/B *trans* junction was determined by the positive Cotton effect for the n-π^* transition (R-band) in the CD spectrum similar to that observed for model compound **11** [7].

The structure proposed for nanuzone was confirmed by partial synthesis from compactone (**10**), a isopimarane diterpene isolated from *V. compacta* [9]. Compactone was dehydrated with HCl–MeOH [9] and the product (**11**) was epoxidized with *m*-CPBA in dichloromethane to afford a diastereoisomeric mixture, whose chromatographic and spectroscopic data showed it to be composed of nanuzone and its C-15 epimer.

11β-Hydroxy-nanuzone (**9**) is a colourless crystalline compound, mp 149–151°, molecular formula $C_{20}H_{30}O_3$ (HRMS). Its spectral data showed it to be closely related to nanuzone.

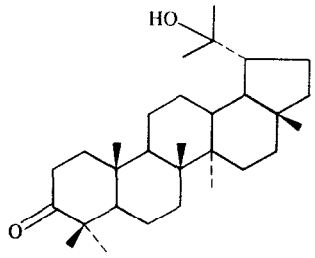
The IR spectrum revealed absorptions for hydroxyl (3350 cm^{-1}) and for a conjugated carbonyl group (1650 cm^{-1}). The UV spectrum was typical for an α,β-unsaturated carbonyl group (λ_{max}^{EtOH} 250 nm, $\log \varepsilon = 3.90$).

The ^1H NMR spectrum of 11β-hydroxy-nanuzone is similar to that of **8** except for the presence of a carbinolic methine signal at δ 4.81 and a deshielded tertiary methyl group signal at δ 1.35. The low field position of the CHOH proton signal suggests it to be allylic. All these data pointed toward a structure similar to that of nanuzone with an additional hydroxyl substituent at C-11. The methine signal at δ 66.1, attributable to C-11, and the methylene signal at δ 41.6, attributable to C-12, are consistent with the proposed structure.

The stereochemistry of the hydroxyl group at C-11 was deduced from the ^1H NMR spectrum of **9** in deuteropiridine. The methyl group at C-20 suffered a downfield shift of δ 0.23 on changing from deuterochloroform to deuteropyridine solution, establishing a syndiaxial relationship with the hydroxyl group.

The structure of **9** was confirmed by epoxidation of **12**, a diterpene isolated from *V. campacta* [3], with *m*-CPBA, in dichloromethane, furnishing a mixture of two isomers identified as 11β-hydroxy-nanuzone and its C-15 epimer.

The structures of nanuzone and 11β-hydroxy-nanuzone were entirely elucidated except for the stereochemistry at C-15, which is currently being investigated.



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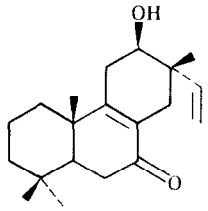

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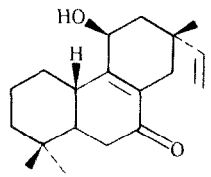

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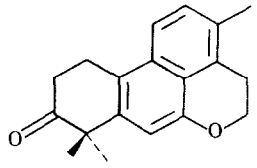
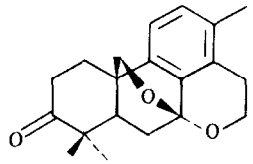
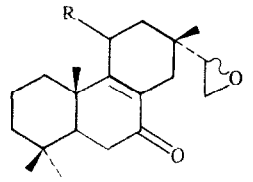


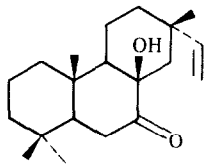
4
5 = Δ^1



6 = Δ^1
7



8 R = H,H
9 R = βOH,H



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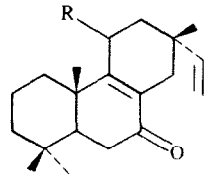

11 R = H,H
12 R = βOH,H

Table 1. ^{13}C NMR chemical shifts of compounds **8**, **9**, **11** and **12** ($CDCl_3$)

C	8	9	11	12
1	35.7[a]	35.4	35.4	35.3[a]
2	18.6	18.6	18.5	18.5
3	41.1	41.1[a]	41.1	41.1
4	33.1	33.4	33.0	33.2
5	50.2	50.9	50.1	51.0
6	35.3[a]	35.4	35.4	35.1[a]
7	199.4	201.1	199.7	201.6
8	128.4	129.7	128.6	130.2
9	165.5	163.0	165.7	163.4
10	39.8	40.8	39.7	40.1
11	22.2	66.1	22.9	67.1
12	30.9[b]	41.6[b]	33.4[a]	44.8
13	31.0	31.9	34.4	35.2
14	31.3[b]	32.4	33.7[a]	34.1[a]
15	57.3	58.1	145.0	144.5
16	44.3	43.8	111.4	111.6
17	22.7	22.9	27.9	28.3
18	32.5	32.7	32.4	32.8
19	21.4	21.6	21.3	21.6
20	17.9	19.4	17.8	19.0

[a, b] Interchangeable values.

EXPERIMENTAL

Mps: uncorr. CC: Merck silica gel (0.05–0.20 mm); TLC: Merck silica gel H, G or $PF_{254+366}$. ^{1}H and ^{13}C NMR spectra were obtained at 100 and 25.2 MHz, respectively, in $CDCl_3$ with TMS as int. standard.

Vellozia nanuzae was collected in Serra do Cipó, State of Minas Gerais, Brazil. Stem, roots and leaf sheaths were cut into small pieces and extracted with hexane, EtOAc and EtOH. The extracts were concd under red. pres. and the residues were chromatographed over silica gel using hexane–EtOAc with increasing amounts of EtOAc as eluent. The hexane extract (7 g) gave a mixture of β-sitosterol and stigmasterol (0.05 g eluted with 5% EtOAc), nanuzone (**8**) (0.155 g, 5% EtOAc), oleic acid (0.02 g, 10% EtOAc); and 11β-hydroxy-nanuzone (**9**) (0.026 g, 20% EtOAc). The EtOAc extract (17 g) yielded 7,16-epoxy-20-nor-1,5,7,9,11,13-cleistanthahexaen-3-one (**5**) (0.022 g, 2% EtOAc), 7,16-epoxy-20-nor-5,7,9,11,13-cleistanthapentaen-3-one (**4**) (0.01 g, 2% EtOAc), a mixture of β-sitosterol and stigmasterol (5% EtOAc), nanuzone (**8**) (0.025 g, 5% EtOAc), (5*R*,7*S*,10*S*)-7α,16;7β,20-diepoxycleistantha-1,8,11,13-tetraen-3-one (**6**) (0.045 g, 5% EtOAc), (5*R*,7*S*,10*S*)-7α,16;7β,20-diepoxycleistantha-8,11,13-trien-3-one (**7**) (0.03 g, 10% EtOAc), 20-hydroxy-lupan-3-one (**1**) (0.072 g, 10% EtOAc), 11β-hydroxy-20-nor-8(9),15-isopimaradien-7-one (**3**) (0.03 g, 10% EtOAc), 12β-hydroxy-8(9),15-isopimaradien-7-one (**2**) (0.14 g, 15% EtOAc), and 11β-hydroxy-nanuzone (**9**) (0.05 g, 20%

EtOAc). From the EtOH extract, were obtained palmitic acid (0.04 g, 10% EtOAc) and caffeic acid (0.02 g, 20% EtOAc). The known compounds were identified by comparison of the spectral data with those of authentic samples.

Nanuzone (**8**). Colourless needles, mp 132–134° from hexane, $[\alpha] = +50$ ($CHCl_3$; *c* 0.78); IR ν_{max}^{KBr} cm^{-1}: 2920, 1660, 1620, 1450, 1370, 1280, 840 and 810; UV λ_{max}^{EtOH} 251 nm ($\log \varepsilon = 4.10$); ^1H NMR: δ 0.86 (3H, *s*), 0.89 (3H, *s*), 0.93 (3H, *s*), 1.10 (3H, *s*) and 2.4–2.8 (3H, *m*); ^{13}C NMR: Table 1; MS *m/z* (rel. int.): 302 $[M]^+$ (100), 287 (7), 271 (26), 269 (27), 245 (30), 243 (46), 161 (31), 160 (39), 149 (48), 147 (50). 135 (28), 134 (29), 133 (30) and 123 (53). Found, *m/z* 302.2234 ($C_{20}H_{30}O_2$ required 302.2245).

Epoxidation of 8(9),15-*isopimaren-7-one* (**11**). *m*-CPBA (34 mg) was added to a soln of **11** (37 mg) in CH_2Cl_2 (10 ml). After stirring for 3 days at 0–5°, the reaction mixture was washed with 5% aq. $NaHSO_3$ (10 ml), 8% aq. $NaHCO_3$ (4 × 10 ml) and brine (2 × 10 ml), dried and concd *in vacuo*. The residue (31 mg) was crystallized from hexane yielding nanuzone together with its C-15 epimer. The ^1H NMR and IR spectra are identical with those of the natural compound.

11β-*Hydroxy-nanuzone* (**9**). Colourless needles, mp 149–151° from hexane and EtOAc (9:1). IR ν_{max}^{KBr} cm^{-1}: 3350 (br), 2910, 1650, 1610, 1450, 1370, 1260, 840 and 810; UV λ_{max}^{EtOH} 250 nm ($\log \varepsilon = 3.90$): ^1H NMR: δ 0.89 (3H, *s*), 0.96 (3H, *s*), 0.98 (3H, *s*), 1.35 (3H, *s*), 2.4–2.8 (3H, *m*) and 4.81 (1H, *m*); ^{13}C NMR: Table 1; MS *m/z* (rel. int.): 318 $[M]^+$ (73), 300 (7), 285 (20), 260 (26), 149 (47), 135 (31), 123 (44) and 41 (100). Found, *m/z* 318.2175 ($C_{20}H_{30}O_3$ requires 318.2194).

Epoxidation of 11β-*hydroxy*-8(9),15-*isopimaren-7-one* (**12**). *m*-CPBA (179 mg) was added to a soln of **12** (208 mg) in CH_2Cl_2 (25 ml). The reaction mixture underwent the same work-up as above. The residue was purified by silica gel CC to give a crystalline product (135 mg) which was identified by ^{13}C NMR as 11β-hydroxy-nanuzone and its C-15 epimer (2:1).

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