TETRACYCLIC TRITERPENES OF BARBACENIA BICOLOR

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Abstract—The isolation of $3\beta_{20}(R)$ -dihydroxydammer-24-ene and 20(R)-hydroxydammar-24-en-3-one from Barbacenia bicolor Mart. is described.

INTRODUCTION

Barbacenia bicolor Mart. (Fam. Velloziaceae) occurs in the sub-tropical regions of South America, especially in the state of Minas Gerais (Brazil).

Hexane extracts of the root and stem furnished, as principal components, two compounds, which are shown in this report to be 3β ,20(R)-dihydroxydammar-24-ene (1) and 20(R)-hydroxydammar-24-en-3-one (2) respectively.

RESULTS

Compound 1 gave a positive test with tetranitromethane [1]. the IR spectrum presented absorption bands for a hydroxyl group, a trisubstituted double bond (at 1660 and 885 cm⁻¹) and a gem-dimethyl group (1350 cm⁻¹).

The NMR spectrum of 1 showed signals for five quaternary methyl groups between 0.80 and 1.00 ppm, a signal at 1.25 ppm for a methyl on the same carbon as an oxygen, two vinyl methyl groups at 1.65 and 1.70 ppm, a double doublet centred at 3.20 ppm (J 6, 10 Hz) for a carbinolic proton α -oriented in a cyclohexane ring [2] and a signal at 5.10 ppm for a vinylic proton. The signal at 0.88 ppm disappeared on the addition of deuterium oxide, showing the presence of a hydroxyl group.

The acetylation of 1 with acetic anhydride and pyridine gave the monoacetate, 3, which presented absorption bands in the IR for a hydroxyl group besides the double bond and an acetate group.

The NMR of 3 showed a double doublet at 4.50 ppm J 6, 10 Hz) with a displacement of 1.3 ppm from the corresponding proton in 2, characteristic of the 3α proton with oxygen (3β) in a 3β -hydroxy-triterpene structure [2].

When 1 was oxidized with Jones reagent [3], a product was obtained whose characteristics (IR, NMR, MS, $[\alpha]_D$, mp, TLC) were identical with 2, isolated directly from the hexane extract, showing that the alcohol 1 and the ketone 2 are both present in the plant.

Compound 2 gave a positive test with tetranitromethane and showed absorption bands in the IR for a hydroxyl, a carbonyl in a six-membered ring (1705 cm^{-1}) and a trisubstituted double bond, besides the gem dimethyl group.

MS analysis of 1, 2 and 3 showed the tetracyclic nature of the substances. Peaks at m/e 249, 207 and 205 indicated the A, B and C rings of the tetracyclic skeleton [4] limiting the choice to the dammarane ring system and excluding euphane or lanostane type skeletons [5]. Peaks at m/e 359, 317, 315 are due to the rupture of the bond between C-17 and C-20 [6]. The peak at m/e127 corresponds to the side chain (C₈H₁₅O) and m/e109 corresponds to the side chain less water (m* at m/e93.5 corresponds to 127–109), (Table 1).

The CMR spectrum of 2 (Table 2) showed a signal at 212.4 ppm for a triterpene possessing a 3-keto [7,8] group. A signal at 74.7 ppm was attributed to C-20 which

Table 1. Mass spectra fragmentation

Fragment	Compounds				
	1	2	3	4	
a	317 (4.0)*	315 (17.8)	359 (6.0)	401 (7)	
b	127 (25.0)	127 (22.4)	127 (15.0)	127 (7.5)	
c	69 (76.0)	69 (92.1)	69 (60.0)	69 (77.0)	
b-18t	109 (100.0)	109 (100.0)	109 (70.0)	109 (100.0)	
d-18†	357 (5.8)	355 (30.7)	399 (4.0)	341 (7.0)	
e	207 (28.5)	205 (26.7)	249 (7.0)	191 (56.0)	

* Relative intensities are given in parenthesis. $\dagger[(M^+-18)-69]$, *m** 299.12, obs.; 299.15 calc. $\ddagger (b-18)$, *m** 93.50, obs.; 93.52 calc.

 Table 2. Chemical shifts in the CMR. Spectrum of 20(R)-hydroxydammar-24-en-3-one

Carbon	Calc.	Obs.	
20	75.26	74.40	
21	27.29	27.29	
3	-	212.4	
24	124.29	124.40	
25	130.56	131.10	
26	24.7	25.5	
27	16.8	17.5	

At 25, 1 MHz ppm; TMS as internal reference; $CDCl_3$ as solvent.

possesses a tertiary hydroxyl group [9], and signals at 124.4 and 131.1 ppm were due to C-24 and C-25 of the side chain [10]. The signals at 17.5 and 25.5 ppm can be attributed to C-27 and C-26 respectively [11] thus characterising the nature of the side chain with a terminal isopropylidene grouping.

The CD curve of 2 gave a weakly negative Cotton effect, similar to dipterocarpol [12] which made it possible to postulate the A and B rings as being *trans*-anti*trans*, having the C-5 hydrogen α -oriented.



Compound 2 gave a mixture of two compounds 4 and 5 in the proportion 1:3, when submitted to Wolff-Kishner reduction [13]. The IR spectra of 4 and 5 gave absorption bands for a hydroxyl group, a trisubstituted double bond and gem-dimethyl but not carbonyl group.

The NMR spectra of 4 and 5 were very similar, the only difference being in the position of a methyl group which appeared at 1.15 ppm in 4 [14] and 1.25 ppm in 5.

Compound 4 was shown to have physical properties identical with data published for the dipterocarpol reduction product [14], indicating that 5 was the C-20 epimer with the (R) configuration.

That 5 is produced from 2 by reduction, and that 4 is produced by reduction and epimerisation was shown by heating 5 with alkali under the same conditions as the Wolff-Kishner reduction (but without hydrazine), only 4 being obtained after the work-up.

The results obtained showed that the two compounds, 1 and 2, have the R configuration at C-20 [15–17] and are epimers of those described by Mills [18].

The epimerisation by base at C-20 is interesting and is felt to include the interaction of the hydroxyl at C-20 with the double bond at C-24 and C-25. Experiments with the double bond absent showed no signs of epimerisation from (R) to (S) taking place. Care was necessary in the isolation of the two compounds (1 and 2) as it was observed that the epimerisation at C-20 from (R) to (S) took place even when the hexane extract was being chromatographed on a silica gel column if the separation was not effected rapidly. The above results are compatible with the structures 1 and 2 with the R-configuration at C-20.

EXPERIMENTAL

Mp's are recorded in a Koffler block; IR spectra in KBr disc; NMR spectra in $CDCl_3$ soln., with TMS as internal standard. CMR spectra in $CDCl_3$ soln., with TMS as internal standard. ORD curve using dioxane as solvent. Column chromatography was on Merck Si gel (0.05–0.20 mm), TLC on Merck Si gel H, G or $PF_{254+366}$.

Extraction. Stem and roots of B. bicolor obtained from the state of Minas Gerais were chopped and dried at ambient temp, then powdered, in a hammer mill. Dried plant material (2 kg) was Soxhlet extracted with hexane for 48 hr. The hexane soln was conc in vacuo to give a crude extract (410 g). Chromatography of the extract (100 g) on a 1.5 kg Si gel column, eluting with C_6H_6 -EtAC (9:1), gave a fraction A (18 g) which was shown to be a mixture of 1 and 2. Purification of fraction A by preparative TLC gave pure 1 and pure 2. Compound 1 was crystallised from hexane to give colourless crystals mp 152–4°. $[\alpha]_D - 11^\circ$ (c, 0.12, CHCl₃); UV λ_{max}^{ErOH} nm(log ϵ) 204 (3.61). IR v_{max}^{KBr} cm⁻¹: 3450, 2930, 1663, 1350, 1028, 885; NMR (CDCl₃): δ 5.10 (1H, m), 3.20 (dd, J 6, 10 Hz), 1.70 (3H, s), 1.65 (3H, s), 0.80 (3H, s). MS m/e: 444.3859 (2%) (M⁺ calcd. for C₃₀H₅₂O₂:444.3850). Compound 2 was crystallised from hexane to yield colourless crystals mp 99-102°, $[\alpha]_D = 50^\circ$ (c, 0.18, CHCl₃). UV λ_{max}^{EtOH} nm(log ϵ): 292 (1.60). IR ν_{max}^{KBr} cm⁻¹: 3450, 1660, 1447, 1350, 1250, 1024, 885. NMR (CDCl₃, 220 MHz): $\delta 5.07$ (1H, m), 2.45 (2H, m), 1.67 (3H, s), 1.65 (3H, s), 1.23 (3H, s), 1.05 (3H, s), 1.02 (3H, s), 0.985 (3H, s), 0.97 (3H, s), 0.91 (3H, s); MS m/e: 442.3808 (58%) M⁺ calcd. for $C_{30}H_{50}O_2$:442.3818). CD curve (c, 0.09 in dioxane) weakly negative Cotton effect $\Delta \epsilon_{325}$ 0.033, $\Delta \epsilon_{295.5} + 0.70$ (a = 30).

Acetylation of 3β ,20(R)-Dihydroxydammar-24-ene (1). To a soln of 3β ,20(R)-dihydroxydammar-24-ene (1, 100 mg) in C₆H₅N (2 ml) was added Ac₂O (2 ml). The mixture was left overnight at room temp, then extracted with CHCl₃ (5 × 20 ml), washed with 1N HCl, neutralized, dried and after solvent evaporation in vacuo gave a crystalline residue (85 mg). Recrystallisation from hexane yielded 3 (45 mg), mp 165-7° [α]_D -9° (c, 0.18, CHCl₃), IR v_{max}^{KBr} cm⁻¹: 3460, 2890, 1710, 1651, 1450, 1350, 1240, 1180, 1015, 885; NMR (in CDCl₃): δ 5.11 (1H, m), 4.52 (1H, dd, J 6, 10 Hz), 2.15 (3H, s), 1.70 (3H, s), 1.65 (3H, s), 1.25 (3H, s), 0.95 (3H, s), 0.93 (3H, s), 0.86 (9H, s).

Jones oxidation of 3β ,20(R)-dihydroxydammar-24-ene (1). Dammarenediol-I (1, 100 mg) was oxidized with CrO₃-H₂SO₄ (8 drops) [3]. After work up a compound was obtained which was shown to be identical by IR, NMR, $[\alpha]_D$, MS and TLC, to 3-keto-20(R)-hydroxydammar-24-ene (2).

Wolff-Kishner Reduction of 2 [13]. The compound 2 (250 mg) was heated at 100° for 2 hr with diethylene-glycol (2.5 ml), KOH (200 mg) and 100% hydrazine hydrate (180 μ l). The mixture was further heated at 200° for 2.5 hr with agitation. After cooling it was poured into 1N HCl (6 ml). The aq. soln was extracted with CHCl₃ (100 ml) which was washed with 5% aq. NaHCO₃ (10 ml), dried and conc *in vacuo* to give a residue of 2 compounds which were immediately chromatographed on prep TLC (hexane-EtAc 4:1 developed 2×), to produce 4 (35 mg, R_f 0.55) and 5 (98 mg, R_f 0.45). The compound 4 showed IR $\nu_{max}^{CRCl_3}$ cm⁻¹: 3530, 2920, 2860, 1450, 1375, 1360, 1200, 1050, 885, NMR (CDCl₃): δ 5.10 (1H, m), 1.70 (3H, s), 1.65 (3H, s), 1.15 (3H, s), 1.00 (6H, s), 9.90 (3H, s), 0.85 (3H, s), 0.80 (3H, s). The compound 5 showed: IR

 $v_{max}^{CHCl_3}$ cm⁻¹: 3560, 2940, 2860, 1455, 1375, 1360, 1220, 1035, 880. NMR: (CDCl₃): δ 5.10 (1H, m), 1.70 (3H, s), 1.65 (3H, s), 1.25 (3H, s), 0.85 (3H, s), 0.80 (3H, s).

Epimerization of 5 to 4. The compound 5 (70 mg) was heated at 200° for 6 hr in diethylene glycol (9 ml) KOH (960 mg), with agitation. After cooling, it was submitted to the same work-up as above, only 4 (42 mg) being obtained (IR, NMR, TLC).

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