NMR Study of the Preparation of 6α,7β-Dihydroxyvouacapan-17β-oic Acid Mannich Base Derivatives

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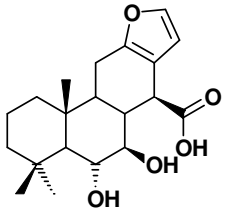
Abstact: *This work presents four new Mannich base compounds obtained by the Mannich reaction of a δ-keto-lactone derivative of 6α,7β-dihydroxyvouacapan-17β-oic acid, a furanoditerpene isolated from the hexane extract of Pterodon polygalaeflorus Benth fruits, which shows anti-inflammatory and analgesic activities. The use of 1D and 2D NMR (COSY, DEPT-135, HMBC, HMQC) spectroscopy made it possible to characterize the new compounds*

Resumo: *Neste trabalho são apresentadas quatro novas bases de Mannich obtidas via reação de Mannich a partir de um derivado δ-ceto-lactônico do ácido 6α,7β-diidroxivouacapan-17β-óico, um furanoditerpeno isolado do extrato hexânico dos frutos de Pterodon polygalaeflorus Benth, que apresenta atividades antiinflamatória e analgésica. O uso de técnicas de RMN 1D e 2D (COSY, DEPT-135, HMBC, HMQC) possibilitou a caracterização dos novos compostos obtidos.*

Introduction

In Brazilian folk medicine, the alcohol extract obtained from the fruits of *Pterodon polygalaeflorus* Benth is used in anti-rheumatic, anti-inflammatory and analgesic preparations. From the hexane extract of the fruits of this plant, furanoditerpene 6α,7β-dihydroxyvouacapan-17β-oic acid (**1;** ADV) was isolated.[1] This compound presents anti-inflammatory and analgesic activities[1,2], as well as activity against the radicle growth of *Sorghum bicolor* L. and *Cucumis sativus* L.[3]



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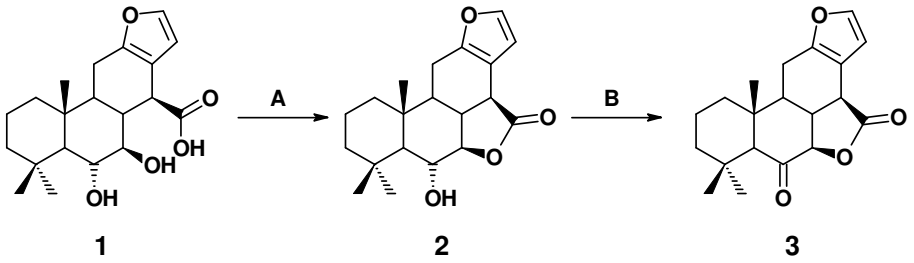
Four new δ-keto-lactone amine derivatives were synthesized so that it could provide some clues about the relationship between the structure and the biological activity and to search for more potent compounds for comparative studies. The synthesis and NMR identification of these compounds are reported.

Experimental

NMR spectra were recorded in $CDCl_3$ on a Bruker DRX 400 *AVANCE* spectrometer. TMS was used as the internal standard. One-dimensional ^1H and ^{13}C NMR spectra were acquired under standard conditions using a direct detection 5 mm $^1H/^{13}C$ dual probe with 90^0 pulse lengths of 11.3 and 8 μs for ^1H and ^{13}C, respectively. The same relaxation delay = 2s was used for all routine experiments. Standard pulse sequences were used for 2D homonuclear and heteronuclear shift correlation spectra, employing a multinuclear inverse detection 5 mm probe with ^1H 90^0 pulse width = 11.3 μs.

To obtain new ADV (**1**) derivatives, 6-α-hydroxyvouacapan-7β,17β-lactone **2** was previously prepared by reaction of **1** with acetic anhydride in tetrahydrofuran at 45 °C in the presence of sodium acetate.[4] The product lactone (**2**) was oxidized by the Swern oxidation[5,6] to 6-oxovouacapan-7β,17β-lactone **3** (Scheme 1).



1 **2** **3**

A - $(CH_3COO)_2O$, CH_3COONa, THF, 45 °C **B -** 1) $(COCl)_2$, DMSO, CH_2Cl_2, N_2, -60 °C 2) Et_3N, r.t.

Scheme 1. Synthetic procedure for preparation of δ-keto-lactone **3**.

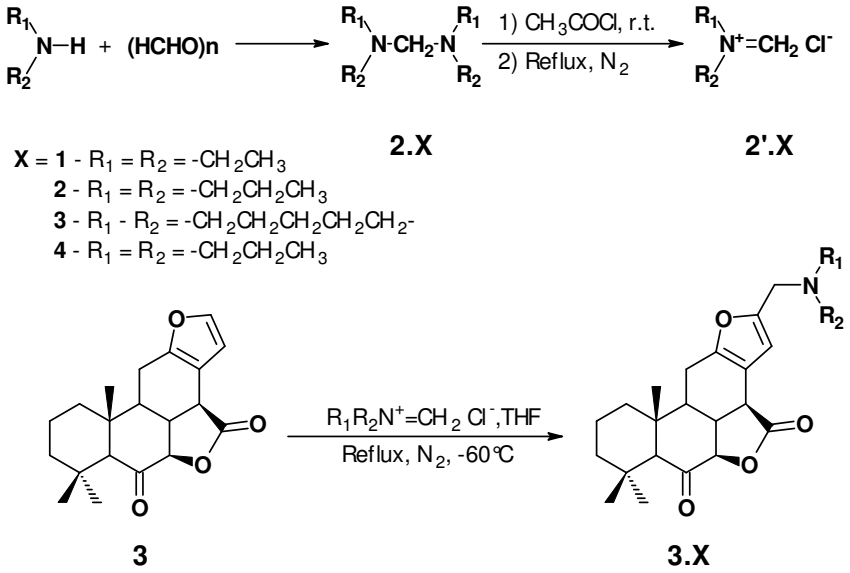
The new compounds - 16-(diethylaminomethyl)-6-oxovouacapan-7β,17β-lactone (**3.1**), 16-(dipropylaminomethyl)-6-oxovouacapan-7β,17β-lactone (**3.2**), 16-(1-piperidinylmethyl)-6-oxovouacapan-7β,17β-lactone (**3.3**) and 16-(dibenzylaminomethyl)-6-oxovouacapan-7β,17β-lactone (**3.4**) - were prepared by a Mannich reaction with preformed iminium salts (Scheme 2).[2] Each compound formation was monitored by ^{1}H NMR. The reaction of the production of each compound was followed by ^{1}H NMR.

Results and discussion

Tables 1 and 2 show ^{13}C and ^{1}H NMR data for compounds **3.1** to **3.4**, respectively. The success of the Mannich reaction was efficiently verified by comparing the signals of the aromatic hydrogens in the NMR spectra of **3** with those of the products **3.1** to **3.4**. This confirmation was possible because the H-16 signal assigned for lactone **3** (δ_H 7.31, *J* 2.0 Hz),[7] which presents a doublet as a result of coupling between H-16 and H-15, was absent in the spectra of compounds **3.1** to **3.4**. In all the ^{1}H NMR spectra of compounds **3.1** to **3.4,** only a single signal between δ_H 6.38 and 6.42 can be observed in the aromatic region, which was attributed to the respective H-15 (see Table 2).

The chemical shift values for C-1 to C-20, corresponding to the vouacapan backbone, are very similar for the four new compounds and compound **3** (Table 1). The main differences among these compounds result from the respective resonances of the methylamino groups at C-16. The C-16 carbons of the four new compounds were deshielded by about 10 ppm relative to that of compound **3** because of the presence of this substituent. Hence, at this position in the molecule the presence of substituents does not cause a shift in the signals of the other neighboring carbon atoms in

compounds **3.1** to **3.4** relative to those of compound **3**. All these facts confirm the success of the Mannich reaction.

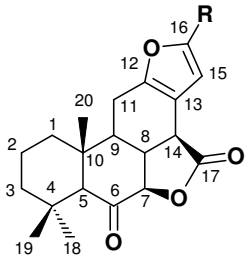


Scheme 2. Synthetic procedure for preparation of Mannich bases derivatives **3.1** to **3.4**

The patterns of the hydrogen signals of the vouacapan backbone of compounds **3.1** to **3.4** are also similar to those of compound **3**.[7] As can be verified in Table 2, the ^{1}H NMR spectra of all these derivatives present complex signals in the region of aliphatic hydrogen resonance between δ_H 1.00 and 3.55, for example, multiplets between 3.32 and 3.55 corresponding to H-14; between 2.13 and 2.32 as a result of overlapping of H-5, H-8, and H-9; between 2.76 and 2.80 due to H-11eq; between 2.57 and 2.62 due to H-11ax. These last two resonances of geminal hydrogens, as well as those of H-5, H-8 and H-9, were assigned with the help of the HMQC experiment. In fact, all the chemical shift assignments for compounds **3.1** to **3.4** were facilitated by employing 2D COSY, NOESY, HMQC, and HMBC techniques. To exemplify, Figure 1 shows some of the more important NOESY correlations for compound **3.2**.

Table 1. ^{13}C NMR data for compounds **3.1** to **3.4** and **3** (100 MHz, $CDCl_3$)

	3.1	**3.2**	**3.3**	**3.4**	**3'**
Atom	δ	δ	δ	δ	δ
1	38.57	38.48	38.70	38.64	38.75
2	17.96	17.87	17.99	17.99	18.02
3	42.53	42.44	42.64	42.58	42.68
4	32.92	32.83	32.95	32.94	32.99
5	63.44	63.34	63.55	63.48	63.61
6	200.96	200.87	200.64	200.81	200.57
7	83.53	83.43	83.52	83.53	83.54
8	49.87	49.78	49.92	49.85	50.04
9	44.98	44.88	45.15	45.08	45.21
10	46.36	46.25	46.35	46.35	46.34
11	21.95	21.86	22.02	21.99	21.94
12	151.19	150.98	151.52	150.93	151.84
13	113.51	113.46	113.80	113.60	113.32
14	42.08	42.00	42.12	42.11	42.11
15	106.47	106.31	107.58	106.55	107.63
16	152.09	152.11	151.82	152.42	142.08
17	171.93	171.84	171.65	171.81	171.57
18	32.85	32.75	32.86	32.88	32.90
19	22.12	22.03	22.11	22.14	22.13
20	15.04	14.94	15.05	15.11	15.05
1'	49.02	49.93	55.46	49.70	
1''	46.69	55.58	-	139.28	
2''	11.62	19.88	54.01	128.78	
3''	-	11.77	25.47	127.11	-
4''	-	-	23.89	126.90	-
1'''	-	-	-	57.78	-



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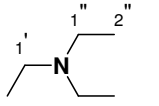

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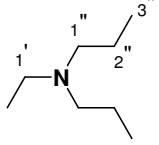

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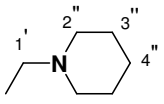

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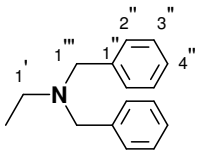
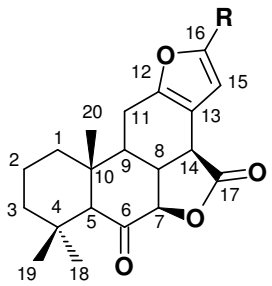


Table 2. ^{1}H NMR data for compounds **3.1** to **3.4** and **3** (400 MHz, $CDCl_3$)

Atom	**3.1** δ, (m), J (Hz)	**3.2** δ, (m), J (Hz)	**3.3** δ, (m), J (Hz)	**3.4** δ, (m), J (Hz)	**3** δ, (m), J (Hz)
H-1ax	1.25-1.32 (m)	1.29 (dt) 13.1, 3.6	1.23-1.32 (m)	1.29 (dt) 13.0, 3.8	1.31 (dt) 13.2, 3.8
H-1eq	1.70-1.78 (m)	1.74 (bd) 13.1	1.57-1.80 (m)	1.73-1.76 (m)	1.76 (ddt) 13.2, 3.2,1.3
H-2ax	1.64 (tq) 13.2, 3.4	1.46-1.75 (m)	1.57-1.80 (m)	1.60 (tq) 13.3, 3.5	1.65 (tq) 13.2, 3.2
H-2eq	1.52 (dqui) 13.2, 3.4	1.41 (bd) 13.1	1.47-1.56 (m)	1.48-1.57 (m)	1.53 (dqui) 13.2, 3.8
H-3ax	1.09-1.20 (m)	1.12 (td) 13.1, 3.3	1.13-(dt) 13.4, 2.5	1.13 (dt) 13.3, 3.5	1.14 (dt) 13.2, 3.8
H-3eq	1.39-1.44 (m)	1.46-1.75 (m)	1.38-1.46 (m)	1.40 (bd) 13.3	1.43 (ddt) 13.2 3.2 1.5
H-5	2.17-2.32 (m)	2.15-2.32 (m)	2.13-2.30 (m)	2.14-2.28 (m)	2.17 - 2.32 (m)
H-7	4.69-4.78 (m)	4.72-4.78 (m)	4.70-4.76 (m)	4.66-4.70 (m)	4.72 - 4.77 (m
H-8	2.17-2.32 (m)	2.15-2.32 (m)	2.13-2.30 (m)	2.14-2.28 (m)	2.17 - 2.32 (m)
H-9	2.17-2.32 (m)	2.15-2.32 (m)	2.13-2.30 (m)	2.14-2.28 (m)	2.17 - 2.32 (m)
H-11ax	2.57-2.65 (m)	2.55-2.65 (m)	2.56-2.67 (m)	2.55-2.62 (m)	2.57 - 2.66 (m
H-11eq	2.76-2.82 (m)	2.73-2.83 (m)	2.73-2.83 (m)	2.74-2.80 (m)	2.75-2.84 (m)
H-14	3.38-3.43 (m)	3.36-3.47 (m)	3.33-3.55 (m)	3.32-3.38 (m)	3.36 - 3.44 (m)
H-15	6.38 (s)	6.42 (s)	6.41 (s)	6.38 (s)	6.58 (d) 2,0
H-18	0.98 (s)	0.98 (s)	0.98 (s)	0.98 (s)	0.99 (s)
H-19	1.36 (s)	1.34 (s)	1.35 (s)	1.36 (s)	1.36 (s)
H-20	0.99 (s)	0.97 (s)	0.98 (s)	0.98 (s)	1.00 (s)
H-1'	3.60 (s)	3.69 (s)	3.33-3.35 (m)	3.56 (s)	-
H-1''	2.54 (q) 7.2	2.48 (t) 7.7	-	-	-
H-2''	1.07 (t) 7.2	1.46-1.75 (m)	2.45 (bs)	7.38 (d) 7.2	-
H-3''	-	0.88 (t) 7.3	1.57-1.80 (m)	7.30 (t) 7.2	-
H-4''	-	-	1.38-1.46 (m)	7.22 (tt) 7.2, 1.4	-
H-1'''	-	-	-	3.61 (s)	-
H-16	-	-	-	-	7.31 (d) 2,0

bd= broad doublet, bs= broad singlet, d= doublet, dqui= double quintet dt = double triplet, ddt= double double triplet, m= multiplet, q= quartet, s= singlet, t= triplet, tq- triple quartet, tt= triple triplet



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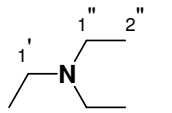

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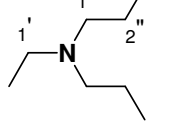

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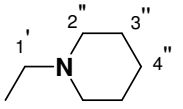

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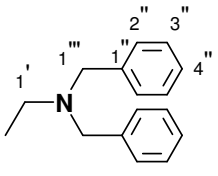

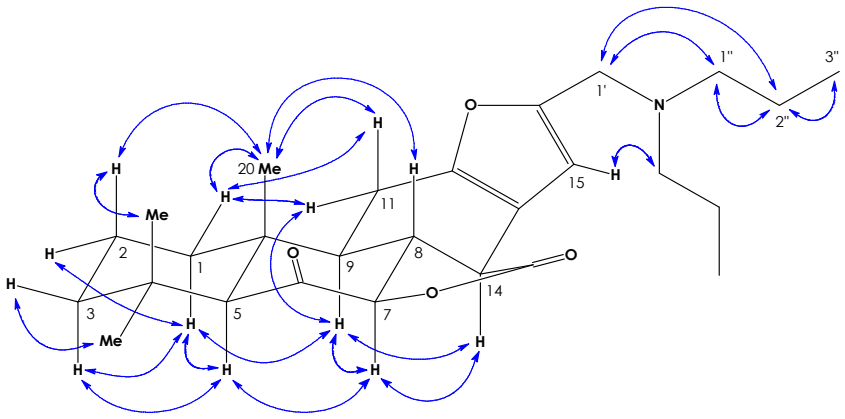

Figure 1. Selected NOESY correlations for **3.2**.

Conclusion

The simplicity of the ^{1}H NMR spectra in the region of aromatic hydrogen resonance of the compounds obtained in this work allowed the Mannich reaction to be monitored by NMR spectroscopy. The ^{1}H and ^{13}C NMR spectra of compounds **3.1** to **3.4** were similar to those of compound **3,** except for new signals corresponding to 16-N-dialkylmethylene groups and the absence of signals corresponding to one of the aromatic hydrogens (H-16). These results confirm the preparation of the new compounds.

Acknowledgements

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