Synthesis of 6α, 7β-di-hydroxyvouacapan-17β -oic acid Derivatives. Parte I: Hydroxamic Acid and Amide Derivatives

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O ácido 6α,7β-di-hidroxivouacapan-17β-óico, isolado dos frutos de *P. polygalaeflorus* Benth apresentou atividade anti-inflamatória e analgésica. Com o objetivo de reunir dados no sentido de elucidar o mecanismo de ação biológica dos bouacapanos, de aumentar sua potência como agente anti-inflamatório e analgésico e a sua tolerância pelo organismo, foram sintetizados ácidos hidroxâmicos (IV e VII) e amidas (III, V e VI) derivados. Os dados espectrométricos no IV, de RMN ^{1}H e ^{13}C e de massa são apresentados e as rotas sintéticas utilizadas são discutidas[1].

6α,7β-Di-hydroxyvouacapan-17β-oic acid, isolated from *P. polygalaeflorus* Benth fruits, possesses anti-inflammatory and analgesic properties. In order to gather data for elucidation of the biological mechanism, to improve the anti-inflammatory and analgesic activities and its organic tolerance, hydroxamic acids (IV and VII) and amides (III, V and VI) derivatives were obtained. The IR, ^{1}H and ^{13}C NMR and Mass spectrometric data are presented and the synthetic routs discussed[1].

Key words: *6α,7β-di-hydroxyvouacapan-17β-oic acid; anti-inflammatory.*

Introduction

The alcoholic extract of "Sucupira branca" seeds (*Pterodon*) has a folk use in Brazil applied to the treatment of "rheumatism" and throat infections[2,3].

Three linear diterpenes and four furan-diterpenes were isolated from *Pterodon polygalaeflorus* Benth[4-6]. One of them, 6α,7β-di-hydroxyvouacapan-17β-oic acid (ADV) has displayed anti-inflammatory and analgesic activities[3,7]. The vouacapan anti-inflammatory activity seems not to be related to fatty acid cyclo-oxygenase inhibition.

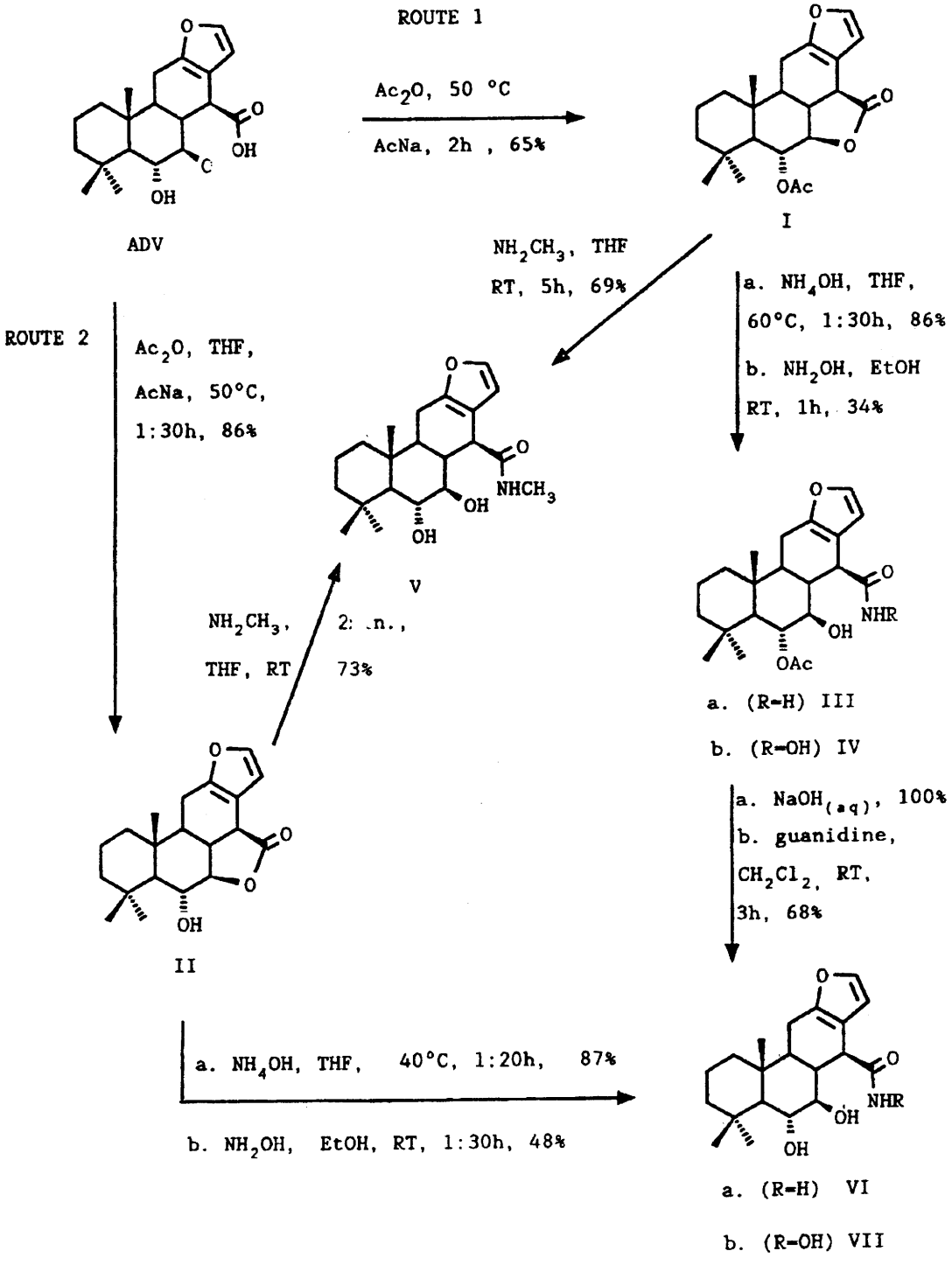
The synthesis of several ADV derivatives[1,8-11] is thus important in order to elucidate the mechanism of voua-capan action as well as to improve it's activity and drug tolerance.

This work had as its main objective the synthesis of ADV nitrogenated derivatives (Scheme 1), to be sent for biological activities studies.

Experimental

Routine experimental procedures and instruments for physical measurements[1]: ^{1}H and ^{13}C NMR spectra were recorded at 80 and 20 MHz, respectively, on a Brucker AC-780). The * means that the signal intensity decreases with D_2O addition and (+) or (-) indicate whether the signal is in the positive or in the negative phase in Dept 135 ^{13}C NMR spectra. Mass spectra were obtained using electron ionization at 70eV on a Varian Mat 311. Infrared spectra were recorded using KBr discs on a Shimadzu IR 408. Melting points were measured on a Mettler FP52. ADV derivatives were prepared by two routes (Scheme 1) according to the described methods below[1].

Preparation of 6α-acetoxyvouacapan-7β,17β-lactone (I): anhydrous sodium acetate (0.54 g , 6.6 mmol) and acetic anhydride (10 ml) were added to ADV (1.00 g, 2.9 mmol).


ROUTE 1
Ac_2O, 50 °C
AcNa, 2h , 65%
ADV
I
NH_2CH_3, THF
RT, 5h, 69%
a. NH_4OH, THF,
60°C, 1:30h, 86%
b. NH_2OH, EtOH
RT, 1h, 34%
ROUTE 2
Ac_2O, THF,
AcNa, 50°C,
1:30h, 86%
V
NH_2CH_3,
THF, RT
2: .n.,
73%
a. (R=H) III
b. (R=OH) IV
a. $NaOH_{(aq)}$, 100%
b. guanidine,
CH_2Cl_2, RT,
3h, 68%
II
a. NH_4OH, THF, 40°C, 1:20h, 87%
b. NH_2OH, EtOH, RT, 1:30h, 48%
a. (R=H) VI
b. (R=OH) VII

Scheme I

The reaction mixture was submitted to mild heating for 2 hours and then was poured onto crushed ice. The mixture was filtered and the solid was washed with water and recrystallized with dichloromethane and n-hexane. White crystals were obtained (0.70 g, 1.9 mmol, 65% yield): m.p.= 273.2-276.4 °C; IR $\bar{\nu}$ (cm^{-1})= 1810, 1730; MS: M+(m/z)= 372 D; ^1H NMR ($CDCl_3$) δ= 1.00(s, 3H, CH_3), 1.08(s, 6H, $2CH_3$), 1.0-2.4 (m, 8H, H1,2,3,5,9), 2.11(s, 3H, $CH_3CO_2^-$), 2.4-2.7(m, 3H, H8, 11), 3.1-3.3(dd, J=3 and 13 Hz, 1H, H14), 4.0-4.3 (dd, J=9 and 11 Hz, 1H, H7), 5.3-5.6(dd, J=9 and 11Hz, 1H, H6), 6.57(d, J=1.8 Hz, 1H, H15), 7.29(d, J=1.8 Hz, 1H, H16); ^{13}C NMR ($CDCl_3$) δ= 15.46 (+), 17.97 (-), 21.84 (-), 22.90 (+), 33.80, 36.49 (+), 39.13 (-), 41.18, 41.65 (+), 44.11 (-), 44.23, 45.71 (+), 57.24 (+), 71.77 (+), 85.06 (+), 107.72 (+), 113.59, 141.75 (+), 152,13, 169.64 (+), 173.10.

Preparation of 6α-hydroxyvouacapan-7β,17β-lactone (II): to a solution of ADV (1.00 g, 2.,9 mmol, 5 ml THF) was added acetic anhydride (0.3 ml, 3.1 mmol) and anhydrous sodium acetate (0.54 g, 6.6 mmol). The reaction mixture was submitted to mild heating for 1.5 h and then it was poured onto crushed ice and filtered. The solid was washed with water and recrystallized with ethanol. White crystals were obtained (0.82 g, 2.49 mmol, 86% yield): m.p.= 226.1-227.9 °C; IR $\bar{\nu}$ (cm^{-1})= 3500, 1770; MS: M^+(m/z)=330D; ^1H NMR (Py-D_5) δ = 0.87 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.52(s. 3H, CH_3), 0.8-2.4(m, 8H, H1,2,3,5,9), 2.4-2.6(m, 3H, H8,11), 3.3-3.6(d, 1H, H14), 4.2-4.4(m, 2H, H7,6), 4.6-4.9(bb,1H*, OH), 6.78 and 7.51(d,2H,H15,16); ^{13}C NMR (Py-D_5) = 15.55 (+), 18.48 (-), 22.08 (-), 23.09(+), 34.40, 37.48(+), 39.32(-). 40.45. 41.96(+), 44.31(+,-), 44.89 (-), 46.06(+), 58.26(+), 71.34(+), 88.82(+), 108.24(+), 114.47, 141.96(+), 152.94, 174.60.

Preparation of 6α-acetoxy-7β-hydroxyvouacapan-17β-hydroxamic acid (IV): To a solution of potassium hydroxide (0.32 g, 5.7 mmol) in 50 ml ethanol was added hydroxylamine hydrochloride (0.39 g, 5.6 mmol). This mixture was stirred until neutralization. then 0.5 g (1.35 mmol) of lactone I was added, this mixture was stirred at room temperature for 1 hour. After that time the reaction mixture was poured onto crushed ice, filtered and the solid was washed with water and recrystallized with dichloromethane and diethyl ether. White crystals were obtained (0.18 g, 0.45 mmol, 34% yield): m.p.= 232.0-234.5 °C, IR $\bar{\nu}$ (cm^{-1}): 3600-3100, 1710, 1670; MS: M^+(m/z)= 405 D; 1H NMR (Py-D_5) = 0.81(s, 3H, CH_3), 1.04 and 1.06(s, 6H, $2CH_3$), 0.6-1.7(m, 8H, H1,2,3,5,9), 2.11(s, 3H, CH_3-CO_2-), 1.7-2.5(m, 2H, H8,11), 2.6-3.2(m, 1H, H11), 3.4-3.8(m, 2H, H7,14), 5.5-5.9(dd, J=9 and 13 Hz, and bb, 3H*, H6,OH,NH), 6.49 and 7.42 (d, J<2 Hz, 2H, H15,16), 10-13 (bb, 1H*, OH); ^{13}C NMR (Py-D_5) δ = 14.89(+), 18.67(-), 22.08(+), 22.49(+,-), 33.34, 36.17(+), 38.62, 39.35(-), 42.34(+), 43.91(-), 46.59(+), 48.85(+), 55.18(+), 76.85(+), 81.08(+), 109.68(+), 116.56, 141.38(+), 151.21, 170.86, 182.82.

Preparation of 6α,7β-dihydroxyvouacapan-17β-hydroxamic acid (VII): Method A: to 2 ml of methanol was added sodium (0.006 g, 0.26 mmol) and guanidine hydrochloride (0.024g, 0.25 mmol). This mixture was added to a solution of IV (0.50 g, 1.2 mmol) in dichloromethane (25 ml). The reaction mixture was stirred for 3 hours at room temperature and then was poured onto crushed ice. The pH was adjusted to 6.5 by addition of acetic acid. This mixture was concentrated, filtered and the solid was washed with water and recrystallized with methanol and diethyl ether. White crystals were obtained (0.30 g, 0.83 mmol, 68% yield). Method B: Hydroxylamine hydrochloride (5.00 g, 52.4 mmol) was added to a stirred solution of potassium hydroxide (4.02 g, 71.7 mmol) in ethanol (60 ml). To this mixture was added lactone II (6.00 g, 18.2 mmol). After 1.5 h at room temperature, the reaction mixture was poured onto crushed ice and filtered. The solid was washed with water and recrystallized with methanol and water. White crystals were obtained (3.2 g, 8.8 mmol, 48% yield): m.p.= 158.7-159.0 °C; IR $\bar{\nu}$ (cm^{-1})= 3600-3100, 1675, 1640; MS: M^+ (m/z)= 363D; ^1H NMR (Py-D_5) δ = 0,85(s, 3H, CH_3), 1.15(s, 3H, CH_3), 1.47(s, 3H, CH_3), 0.8-1.7(M, 8H, H1,2,3,5,9), 2.10-2.54 (m, 2H, H11,8), 2.66-3.07 (m,1H,H11), 3.4-3.9 (m, 2H, H7,14), 3.9-4.2(m, 1H, H6), 6.49 (d, 1H, H15), 6.3-7.4(bb, 4H*, OH,NH), 7.39(d,1H,H16); ^{13}C NMR (Py-D_5) δ= 15.83(+), 18.95(-), 22.49(-), 22.71(+), 33.89, 37.18(+), 38.54, 39.70(-), 41.49(+), 44.16(-), 44.16(-), 46.00(+), 49.13(+), 56.27(+), 74.24(+), 83.57(+), 109.71(+), 116.42, 141.25(+), 151.51, 173.35.

Preparation of 6α-acetoxy-7β-hydroxyvouacapan-17β-amide (III): To a solution of lactone I (0.2 g, 0.54 mmol) in THF (8 ml) was added ammonium hydroxide (5 ml, 40 %). The reaction mixture was stirred at 60 °C for 1.5 h and then poured onto crushed ice and filtered. The solid was washed with water and recrystallized with dichloromethane, THF and diethyl ether. White crystals were obtained (0.18g, 0.46 mmol, 86% yield): m.p.= 247-252 °C; IR $\bar{\nu}$ (cm^{-1})= 3650-3100, 1735, 1660, 1615; MS: M^+(m/z)= 389 D; ^1H NMR (Py-D_5) δ = 0.83(s, 3H, CH_3), 1.03(s, 3H, CH_3), 1.07(s, 3H, CH_3), 1.0-1.7(m, 8H, H1,2,3,5,9), 2.11(s, 3H, CH_3-CO_2^-),1.8-2.6(m, 2H, H11,8), 2.6-3.2(m, 1H, H11), 3.3-3.9(m, J=8 Hz, 2H,H7,14), 4.5-5.1(bb,1H*, OH), 5.5-5.9(dd, J=8 and 13 Hz, 1H, H6), 5.8-6.3(bb, 1H*, NH), 6.62(d, J=1.6 Hz, 1H, H15), 7.47(d, J+1.6 Hz, 1H, H16), 8.0-8.5(bb, 1H*, NH); ^{13}C NMR (Py-D_5) ν= 15.00(+), 18.65(-), 22.078(+), 22.50(+,-), 33.31, 36.17(+), 38.64, 39.37(-), 42.58(+), 43.91(-), 48.87(+), 49.00(+), 55.21(+), 76.80(+), 81.13(+), 109.87(+), 116.38, 141.35(+), 151.12, 170.89, 177.84.

Preparation of 6α, 7β-dihydroxyvouacapan-17β-amide (VI): Method A: To a solution of lactone I (0.2 g, 0.54 mmol) in THF (10 ml) was added ammonium hydroxide (5ml, 40%). The same procedure for the preparation of III was followed. After 1:5 h of reaction, an aqueous solution of sodium hydroxide (5 ml, 5%) was added to the reaction mixture under stirring at room temperature. Then, the solid was washed with water and recrystallized with benzene and THF. White crystals were obtained (0.16 g, 0.46 mmol., 86% yield). Method B: To a solution of lactone II (0.50 g, 1.52 mmol) in THF (10 ml) was added ammonium hydroxide (5 ml 40%). The mixture reaction was stirred at 40°C for 1:20 h and them it was spilled into pricked ice. The product was separated by filtration, washed with water and recrystallized with benzene and THF. White crystals

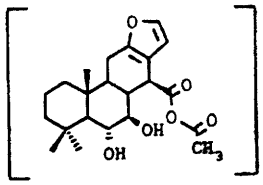
were obtained (0.46 g, 1.32 mmol, 87% yield): m.p.= 141-142°C; IR $\bar{\nu}$ (cm^{-1}) δ = 3650-3100, 1680-1600; MS: M^+(m/z)= 347 D; ^1H NMR (Py-D_5) δ = 0.87(s,3H,CH_3), 1.16 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 0.7-1.8 (m, 8H, H1,2,3,5,9), 2.0-3.0 (m, J=10 and 11 Hz, 3H, H11,8), 3.4-4.2(m, J=9 and 11Hz,3H, H14,6), 4.6-6.0(bb, 3H*, OH, NH), 6.61(d, J=1.6 Hz, 1H, H15), 7.45(d, J=1.6 Hz, 1H, H16), 8.32(bb, 1H*, NH); ^{13}C NMR (Py-D_5) δ =15.65(+), 18.90(-), 22.43(-), 22.68(+), 33.83, 37.11(+), 38.42, 39.65(-), 41.91(+), 44.1(-), 48.63(+), 49.07(+), 56.16(+), 74.26(+), 83.52(+), 109.87(+), 116.29, 141.24(+), 151.11, 178.47.

Preparation of 6α, 7β-dihydroxyvouacapan-17β-methylamide(V): Method A: To a solution of lactone I (0.21g, 0.57 mmol) in THF (10ml) was added methylamine (1 ml, 40%). The reaction mixture was stirred at room temperature for 5 h and then poured onto crushed ice. The product was separated by filtration, washed with water and recrystalized with THF and diethyl ether. White crystals were obtained (0.14 g, 0.39 mmol, 69% yield). Method B: To a solution of lactone II (4.0 g, 12.12 mmol) in THF (50 ml) was added methylamine (2.0 ml, 40%). The reaction mixture was stirred at room temperature for 2 minutes. A white precipitate was formed. The reaction mixture was poured onto crushed ice and filtered. The solid was washed with water and recrystallized with THF and diethyl ether. White crystals are obtained (3.19 g, 8.85 mmol, 73% yield); m.p.= 241.6-243.2°C; IR $\bar{\nu}$ (cm^{-1})= 3650-3050, 1680-1610; MS: M^+(m/z)= 361D; ^1H NMR (Py-D_5) δ= 0.84(s, 3H, CH_3), 1.08(s, 3H, CH_3), 1.47(s, 3H, CH_3), 0.8-1.7(m, 8H, H1,2,3,5,9), 2.0-3.2(m, 3H, H8,11), 2.97-3.04(d, J=5.7 Hz, 3H,CH_3-N), 3.5-3.8(m, 2H, H7,14), 3.8-3.04(d, J=5.7 Hz, 3H, CH_3-N), 3.5-3.8(m, 2H, H7,14), 3.8-4.2(m, 1H, H6), 4,5-5.6(bb, *, OH), 6.53(d, 1H, H15), 7.48(d, 1H, H6), 4,5-5.6(bb, *, OH), 6.53(d, 1H, H15), 7.48(d, 1H, H16), 8.56(dbb, 1H*, NH); ^{13}C NMR (Py-D_5) δ= 15.63(+), 18.91(-), 22.48(-), 22.66(+), 26.07(+), 33.87, 37,12(+), 38,42, 39,65(-), 4.98(+), 44.10(-), 49.75(+), 56,14(+), 74.31(+), 83,53(+), 109.71(+), 116.50, 141.32(+), 151.31, 176.21.

Results and Conclusions

The nitrogenated derivatives of ADV were obtained via two synthetic routes (Scheme 1), some of the derivatives being novel (II, IV, V and VII).

The lactones I[1,2] and II were obtained by the reaction of ADV and acetic anhydride in the presence of anhydrous sodium acetate, under diferent conditions.



The lactonization mechanism probably involves the intramolecular nucleophilic attack of C-7 hydroxy to C-17 carbonyl with the lost of H_2O. The equilibrium is displaced in favour of product formation by the acetic anhydride.

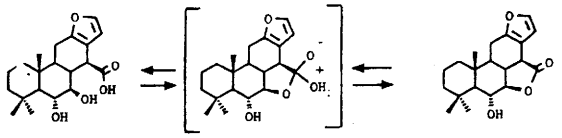
Another possibility is via the mixed anhydride intermediate:

When acetic anhydride is in excess, the hydroxyl at C-6 is also acetylated.The lactone II was obtained in a better yield than lactone I in a faster reaction (Scheme 1). The nitrogenated derivatives of ADV were obtained by the reaction of lactones I and II with nucleophilic reagents (Scheme 1). The basic mechanism in all cases is the nucleophilic attack of nitrogen at the lactone carbonyl. In this way, derivatives V, VI and VII were obtained (Scheme 1) in one step from lactone II in good yields (73, 87 and 48%). In the reactions with lactone I, a selective attack of hydroxylamine at the lactone carbonyl was observed, leading to the acetylated hydroxamic acid IV (34% yield).

The acetylated amide III[1,12] was obtained by the reaction of lactone I and ammonium hydroxide in THF (86% yield). This reagent was also selective as it is less nucleophilic than the others used. We could not detect the presence of VI after short reaction times. However, if the reaction was allowed to continue, a secondary product could be observed by thin layer chromatography(TLC). On the other hand mehtylamine is a stronger nucleophile and it attacked the lactone carbonl as well as the acetate carbonyl giving directly the non-acetylated amide V. However, the reaction of methylamine with lactone II showed a better yield than with lactone I and was 150 times faster.

As mentioned previously, the secondary product formed in the reaction of I and ammonium hyroxide could be the amide derivative VI [1,12]. However the desacetylation reaction with ammonium hydroxide was very slow. So, aqueous sodium hydroxide solution was added to the reaction mixture and we could observe by TLC the consumption of III and the intensification of the second spot. This product was indentified as the derivative XI (86% yield).

The derivative IV was de-acetylate by guanidine, yielding VII (68%). Guanidine[13] is a good nucleophile but weaker and more selective than sodium hydroxide. All reactions with lactone II were faster than with lactone I. This fact confirms that the preferential site of nucleophilic attack is the lactone carbonyl. The opening



$H_2O + (CH_3CO)_2O \longrightarrow 2CH_3CO_2H$

of the lactone ring must also lead to a decreasing in the molecular structure tension and there is also a positive entropic effect. Besides the rapidity of the reactions with lactone II and the reduced number of synthetic steps, route 2 has presented better total yields.

The derivatives obtained were submited for biological studies and the results will be published elsewhere[7].

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References

1. This work is part of the MSc Thesis of M. M. M. Rubinger, *Síntese de Derivados Nitrogenados do Ácido 6α,7β-dihidroxivouacapan-17β-óico (ADV)*, Departa- mento de Química - ICEx, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil (1991)
2. E. A. Nunam, *Estudo da Atividade Antiinflamatória de Furano-diterpenos isolados do Pterodon polyga-laeflorus Benth e de Alguns de Seus Derivados* (Msc Thesis- Departamento de Farmacologia - ICB - Universidade Federal de Minas Geais, Belo Horizonte, Brazil) (1985).
3. E.A.Nuna, D.Piló-Veloso, R.M.M.Turchetti-Maia and D.L.Ferreira Alves, *Braz.J.Med.Exp.Sc.* 15(6), 450 (1982).
4. W.B.Mors, M.F.Santos Filho, H.J. Monteiro, B. Gilbert and J. Pellegrino, *Science* 157, 950-951 (1967).
5. J.R.Mahajan and M.B.Monteiro, *J.Chem.Soc. (Perkin I)* 57, 52s0-525 (1973).
6. M. Fascio, W. B. Mors, B. Gilbert, J. R. Mahajan, M. B. Monteiro, D. dos Santos Filho and W. Vichnewski, *Phytochemistry* 15, 201-203 (1976).
7. D. L .Ferreira-Alves, J. N. Francischi, S. M.S.Pesquero, C. V. Silva, J. L. D.Costa, A.S.F.Porto, M.M.M. Rubin- ger, H.H.Santos, G.M.Stefani and D.Piló-Veloso, *Relação Estrutura-Atividade (Analgésico-Anti- Edematogênica) de Furano-diterpenos do* P. polyga-laeflorus *Bent e Dreivados*, V Reunião Anual da Federação de Sociedades de Biologia Experimental - Caxambu, MG, Brazil (1990).
8. J. M. Moreira, *Síntese de Derivados Glicosilados do Ácido 6α,7β-di-hidroxivouacapan-17β-óico* (Msc Thesis, Departamento de Química, ICEx, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil)(1990).
9. J. M. Moreira, D. Piló-Veloso, G. M. Stefani and D. L. Ferreira Alves, *Ciência e Cultura,* supl.40 (7), 493 (1988).
10. T. M.Sacramento, G. M. Stefani, D. Piló-Veloso and D. L. Ferreira-Alves, *Sintese de Derivados de Furano- Diterpenos I. Obtenção e Caracterização de 6α, 7β-dihidroxivouacapan-17β-oato de etila.* IV Encontro Regional da SBQ-MG, Caxambu, Brazil, Proceedings 34 (1990).
11. C. R. A. Maltha, G. M. Stefani, D. Piló-Veloso and D. L. Ferreira-Alves, *Síntese de Derivados de Furano- Diterpenos II. Obtenção e Caracterização de N-ciclo-exil-6α, 7β-dihidroxivouacapan-17β-amida,* IV Encontro Regional da SBQ-MG, Caxambú, Brazil, Proceedings 35 (1990)
12. E. V. Marques, G. M. Stefani, D. Piló-Veloso e D. L. Ferreira-Alves, *Ciência e Cultura,* 40(7), 492 (1988).
13. N. Kunesh, C. Miet and J. Poisson, *Tetrahedron Letters* 28(31), 3569-3572 (1987).