Iridoids and Triterpenes from *Himatanthus phagedaenica:* The Complete Assignment of the ^{1}H and ^{13}C NMR Spectra of Two Iridoid Glycosides

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Do extrato etanólico do caule de *Himatanthus phagedaenica* foram isolados e identificados uma mistura dos triterpenos α-amirina, β-amirina e lupeol acetilados, β-sitosterol e três iridóides lactônicos: plumericina, alamandina e isoplumericina. Do extrato etanólico-aquoso acetilado foram isolados dois iridóides glicosídicos: octa-O-actilplumeride cumarato glicosídico e penta-O-acetilplumeride glicosídico em uma mistura com sacarose acetilada. As determinações estruturais foram realizadas através da análise de dados espectrométricos. Os assinalamentos dos deslocamentos químicos dos átomos de hidrogênio e carbono (^{13}C) dos iridóides glicosídicos apoiaram-se na análise de resultados obtidos por experiências APT, ^{1}H x ^{1}H-HOMOCOSY e ^{1}H x ^{13}C-HETCOSY ($^{1}J_{CH}$ and $^{n}J_{CH}$; n=2 e 3-COLOC).

From the ethanolic extract of the stem of *Himatanthus phagedaenica* were isolated and identified an acetylated mixture of the triterpenoids α-amyrin, β-amyrin and lupeol, in addition to β-sitosterol and three sesquiterpenic iridoid lactones: plumericin, allamandin and isoplumericin. From the acetylated aqueous ethanolic extract were isolated two iridoid glycosides and penta-O-acetyl plumeride glucoside mixed with octaacetylated sucose. Structural determination were made by spectrometric data. The ^{1}H and ^{13}C NMR spectra of the two iridoid glycosides have been assigned using APT ^{1}H x ^{1}H-HOMOCOSY and ^{1}H x ^{13}C-HETCOSY ($^{1}J_{CH}$ and $^{n}J_{CH}$, n = 2 and 3, COLOC)

Key Words: Himatanthus phagedaenica; *Apocynaceae; ^{1}H and ^{13}C NMR*

Introduction

Himatanthus phagedaenica (Mart) Woodson, family Apocynaceae, is a plant which grows in Northeastern Brasil and is reputed by its use as an anti-helminthic[1]. In this paper we report the chemical investigation of a specimen of this species and the complete assignments of the ^{1}H and ^{13}C NMR spectra of the two iridoid glycosides. The assignments found in the literature were either incomplete or uncertain, so a two-dimensional (2D) NMR study was undertaken to provide complete ^{1}H and ^{13}C assignments.

Experimental

General experimental procedures. Melting points were determined using a Kofler hot-stage instrument and are uncorrected. IR spectra were measured on a Perkin-Elmer 467 spectrometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ and C_6D_6 solutions, using TMS as internal standard, employing a Varian EM-360 (60 MHz), Varian FT-80 (^1H, 80 MHz; ^{13}C, 20 MHz), Varian XL-100 (^1H, 100 MHz; ^{13}C, 25.2 MHz), Bruker AC-200 (^1H, 200 MHz) and Bruker ACE-300 (^1H, 300 MHz; ^{13}C, 75 MHz). Low resolution mass spectra were obtained on a MICRO MASS-12 instrument operating at 70 eV.

Isolation of constituents. Stem of a specimen of *Himatanthus phagedaenica* (Mart) Woodson (collected in João Pessoa, Paraiba State, by botanist Maria de Fátima Agra, Universidade Federal da Paraiba, João Pessoa, and identified by Dr. M.M. Plumel of National Museum of Paris), after drying and being reduced to powder (2 kg), was extracted with EtOH at 60°C for 48 h. Solvent was removed under vacuum to yield 100 g of residue. This residue (50 g) was dissolved in EtOH:H_2O (9:1) and extracted with hexane and $CHCl_3$, successively. The hexane, $CHCl_3$ and aqueous solutions were distilled under vacuum, affording residues **A** (20 g), **B** (10 g), and **C** (10 g), respectively. Residue **A** (5 g) was chromatographed on a silica gel (60 g) column and eluted with hexane, hexane-$CHCl_3$ (7.5:2.5), $CHCl_3$, $CHCl_3$-MeOH (9.5:0.5,9:1, 8:2) and MeOH, furnishing 120 fractions of 100 ml each. Fractions 46-113 (0.8 g), eluted with $CHCl_3$-MeOH (8:2), was recrystallized from $CHCl_3$-MeOH (1:1), affording acetylated mixture of the triterpenoids α-amyrin (**1**), β-amirin (**2**) and lupeol (**3**). Residue **B** (10 g) was chromatographed on a silical gel (120 g) column and eluted with solvents as above. A total of 305 fractions of 200 ml each were collected. Fractions 18-54 (0.9 g), eluted with hexane-$CHCl_3$ (1:1), yielded β-sitosterol (0.3 g) after crystallization from $CHCl_3$-MeOH (9.5:0.5); fractions 55-130 (0.6 g), eluted with $CHCl_3$, furnished an additional quantity (0.12 g) of mixture of **1**, **2** and **3** after crystallization from $CHCl_3$-MeOH (1:1); fractions 131-252 (3.5 g), eluted with $CHCl_3$-MeOH (9.5:0.5 and 9:1), was rechromatographed on a silica gel (70 g) column and were eluted 97 fractions (100 ml) with $CHCl_3$ and $CHCl_3$-MeOH (9.55:0.5; 9:1 and 1:1), yielding **4** (0.2 g), after crystallization from $CHCl_3$-MeOH (1:1) of fractions 35-57 (0.9 g) eluted with $CHCl_3$, **5** (0.08 g), after being rechromatographed by prep-TLC ($CHCl_3$-MeOH, 9.2:0.8) and crystallization from hexane-ether-CH_2Cl_2 (1:0.5:0.5) of fractions 58-59 (0.15 g) eluted with $CHCl_3$-MeOH (9.5:0.5), and **6** (0.15 g), after being rechromatographed by prep-TLC(C_6H_6-MeOH, 9:1) and crystallization from hexane-ether (1:1) of fractions 66-89 (0.6 g) eluted with $CHCl_3$-MeOH (9:1). Residue **C** (2.0 g) was acetylated with Ac_2O (10 ml) and pyridine (8 ml). The solution was kept at room temp for 16 h and usual work-up gave a mixture of acetates (2.0 g). This mixture was fractionated on a silica gel column using C_6H_6 and $CHCl_3$ as eluents, successively. The fraction eluted with $CHCl_3$ was evaporated under vacuum and the residue (0.8 g) was chromatographed by prep-TLC(C_6H_6-EtOAc, 9:1), affording **7a** (0.15 g) and **8a** + **9a** (0.13 g), after crystallization from C_6H_6-$CHCl_3$ (2:1) and CH_2Cl_2-MeOH (1:1), respectively.

Acetates of α*-amyrin* (**1**), β*-amyrin* (**2**) *and lupeol* (**3**). The presence of these three compounds in a mixture was revealed by spectral data, mainly ^{13}C NMR through chemical shifts of carbon atoms when compared with those of the corresponding compounds recorded in the literature[2,3].

Plumericin (**4**). Mp 210-212°C ($CHCl_3$-MeOH, 1:1); spectral data and mp in agreement with reported values[4].

Allamandin (**5**). Mp 218-220°C (hexane-ether-CH_2Cl_2, 1:0.5:0.5); lit. mp 212-215°C (MeOH-EtOAc).[5]

Isoplumericin (**6**). Mp 192-194°C (hexane-ether, 1:1); lit.mp 198-200°C (petrol-Et_2O, 3:2)[6].

Octa-O-acetylplumericin coumarate glucoside (**7a**). Mp 102-104°C (C_6H_6-$CHCl_3$, 2:1). Spectral data in agreement with reported values[6]; ^1H and ^{13}C NMR: see Table 1.

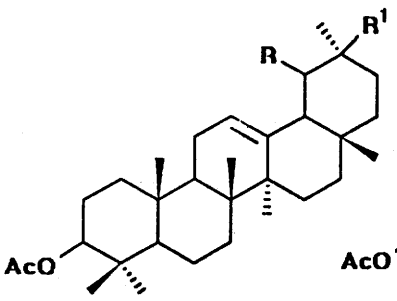
Penta-O-acetylplumeride glucoside (**8a**) + *Octa-O-acetylsucrose* (**9a**). Mp 110-112°C (CH_2Cl_2-MeOH, 1:1), Ir (KBr, max) cm^{-1}: 1780-1710 (carbonyl ester), 1640 (double bond), 1380, 1290-1260, 1140-1020, 950, 910, 760; ^1H and ^{13}C NMR: see Table 1; EIMS *m/z* (rel.int.): 332 (10), 331 (31), 271 (8), 212 (19), 211 (74), 169 (100), 145 (6), 139 (7), 127 (14), 110 (6), 109 (39), 97 (9), 43 (67).

Results and Discussion

The rare iridoids **4-6**, which contain a spiro-lactone ring as an additional feature, were isolated from an ethanolic extract of *Himatanthus phagedaenica* stem, along with an acetylated mixture of the triterpenes α-amyrin (**1**), β-amyrin (**2**) and lupeol (**3**). The structure of these compounds have been determined by spectral properties, which were confirmed by comparison with literature data (see experimental). The iridoid compounds (**4-6**) have been found in species from the genera *Plumeria* and *Allamanda* [6,7]. *Plumericin* (**4**) was also isolated from *Nerium indicum*[6].

The residue obtained from the EtOH extract was acetylated and the iridoids **7** and **8** in a mixture with **9** were isolated as acetyl derivatives (**7a** and **8a** + **9a**). The structures **7a** and **8a** were deduced on the basis of their ^1H NMR spectral data, which were confirmed by comparative analysis with literature values reported for acetyl derivatives of plumeride coumarate and plumeride glucosides, respectively, isolated from *Allamanda cathartica*[6]. Further confirmation of this identity was obtained by ^{13}C NMR spectra (Table 1). The assignment of the signals in these spectra were made on the basis of the observed multiplicities (fully coupled, SFORD - four spectra with different frequencies of irradiation and APT), empirical shift rules[8] and comparison with data from model compound[4] **10**, along with homonuclear (^1H x ^1H - HOMOCOSY) and heteronuclear [^1H x ^{13}C - HETCOSY: modulated with an average value of all $^1J_{CH}$ and of long-range $^2J_{CH}$ and $^3J_{CH}$ (COLOC) coupling constants] shift-correlations 2D NMR experiments[8,9]. THe interpretation of these spectral data for **7a** and **8a** allowed the complete assignments (Table 1) of chemical shifts and multiplicity of the signals corresponding to hydrogen and carbon-13 atoms of the basic skeleton of the aglycone (C-1 to C-15), of the aromatic ring (**7a**) and of the 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl groups. Further, the ^1H and ^{13}C NMR spectra revealed that the aglycone was linked to the 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl group through the oxygen atom of C-1 (Table 1), which was confirmed by the spin coupling ($^3J_{CH}$, long-range modulated with J=10 Hz) between C-1 (δ 92.24) and H-1 (δ 4.82).

After the recognition of the signals corresponding to hydrogen and carbon-13 atoms of iridoid **8a**, which was



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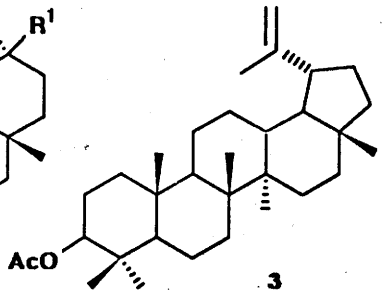
2 R = H, R^1 = Me



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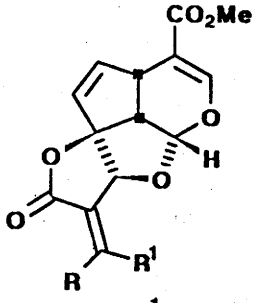
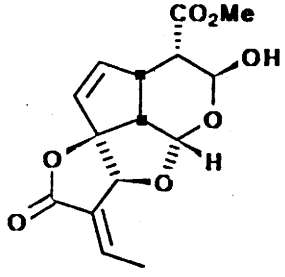
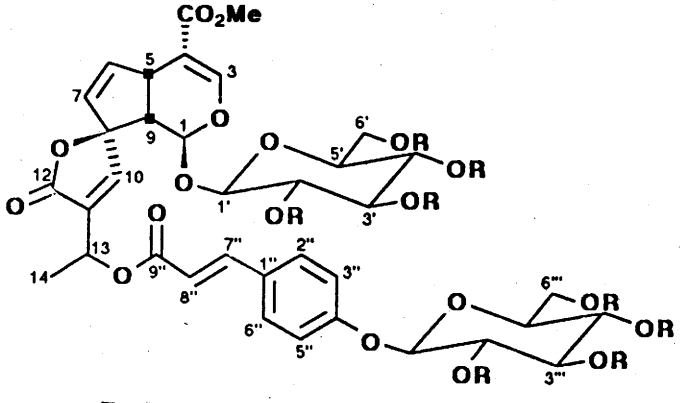


4 R = H, R^1 = Me
6 R = Me, R^1 = H



5



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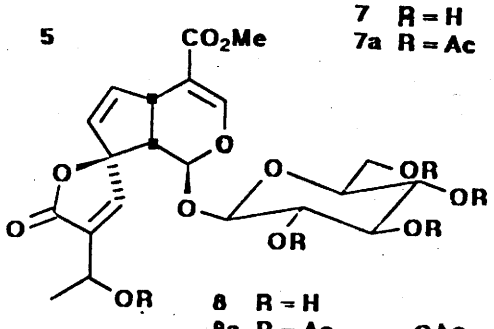
7a R = Ac



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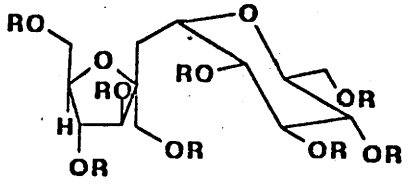
8a R = Ac



9 R = H
9a R = Ac

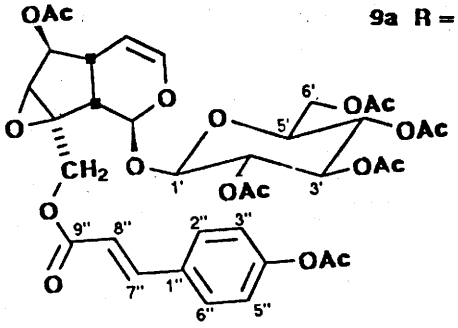


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Table 1. *^1H (300 MHz) and ^{13}C (75 MHz) NMR data of compounds **7a** and **8a** + **9a** (TMS as internal standard, in $CDCl_3$ or C_6D_6) compared with model [4] **10** and sucrose octaacetate [10,11].*

	7a		8a in the 8a + 9a		10		9a in the 8a + 9a				Sucrose octaacetate			
C	δC	δH and J (Hz)	δC	δH and JH (Hz)	δ(C6D6)[a]	δC	C	δ C	δH and J(Hz)	δH(C_6D_6)	δC	δ H	δH(C_6D_6)	
1	92.16(d)	5.10(d,2.3)	92.24(d)	5.08(d,3.3)	5.25		G-1	90.00(d)	5.69(d,3.7)	5.87	89.93	5.69	5.85	
3	149.15(d)	7.38(d,1.4)	149.33(d)	7.39(d,1.5)	7.37		G-2	70.33(d)	4.88(dd,10.4,3.7)	5.05	70.2	4.87	5.02	
4	111.57(s)	-	111.56(s)	-	-		G-3	69.70(d)	5.44(dd,10.4,3.6)	5.82	69.61	5.44	5.81	
5	37.66(d)	3.76(dddd,8.4,3.0,1.6,1.4)	37.85(d)	3.77(dddd,8.3,2.8,1.7,1.5)	3.65		G-4	68.32(d)	5.08(t,9.6)	5.34	68.17	5.08	5.33	
6	138.71(d)	6.43(dd,5.6,3.0)	139.01(d)	6.44(dd,5.6,2.8)	6.21		G-5	68.55(d)	4.29(m)	4.52	68.50	4.28	4.51	
7	129.35(d)	5.45(dd,5.6,1.6)	129.33(d)	5.45(dd,5.6,1.7)	5.03		G-6a	61.82(t)	4.1-4.3	4.35	61.75	4.14	4.36	
8	95.77(s)	-	95.80(s)	-	-		G-6b	-	4.1-4.3	4.42	-	4.28	4.40	
9	48.67(d)	3.17(dd,8.4,2.3)	48.79(d)	3.15(dd,8.3,3.3)	2.92									
10	148.45(d)	6.98(d,1.2)	148.46(d)	6.94(d,1.4)	7.00		F-1a	62.95(t)	4.17(s)	4.31	62.85	4.17	4.28	
11	134.36(s)	-	134.35(s)	-	-		F-1b	-	4.17(s)	4.40	-	4.17	4.37	
12	169.66(s)	-	169.71(s)	-	-		F-2	104.02(s)	-	-	104.02	-	-	
13	65.12(d)	5.78(qd,6.7,1.2)	65.05(d)	5.66(qd,6.7,1.4)	5.80		F-3	75.80(d)	5.45(d,5.6)	5.71	75.68	5.47	5.70	
14	19.32(q)	1.59(d,6.7)	19.22(q)	1.52(d,6.7)	1.50		F-4	75.09(d)	5.37(dd,6.0,5.6)	5.55	74.68	5.47	5.70	
15	166.10(s)	-	166.22(s)	-	-		F-5	79.16(d)	4.20(m)	4.22	79.14	4.21	4.19	
							F-6a	63.66(t)	4.30	4.43	63.63	4.29	4.41	
1'	95.73(d)	4.83(d,8.2)	95.80(d)	4.82(d,8.2)	4.74	96.70	F-6b	-	4.35(dd,12.2,4.5)	4.43	-	4.35	4.41	
2'	70.61(d)	4.96(dd,9.3,8.2)	70.71(d)	4.97(dd,9.4,8.2)	5.25	70.70	OAc[c]	C=O	CH_3			CH_3		
3'	72.33(d)	5.20(t,9.3)	72.40(d)	5.22(t,9.4)	5.40	72.50								
4'	67.98(d)	5.10(dd,9.6,9.3)	68.13(d)	5.10(dd,9.5,9.4)	5.29	68.20	G-2	170.08	20.63	2.10	170.07	20.64[d]	2.10	
5'	72.33(d)	3.70(ddd,9.6,4.5,2.3)	72.33(d)	3.73(ddd,9.5,4.5,2.4)	3.21	72.20	G-3	169.99	20.63	2.02	170.01	20.66	2.02	
6'a	61.53(t)	4.28,(dd,12.5,4.5)	61.63(t)	4.25(dd,12.4,4.5)	4.27	61.30	G-4	169.49	20.63	2.04	169.50	20.61	2.05	
6'b	-	4.09(dd,12.5,2.3)	-	4.11(dd,12.4,2.4)	3.90	-	G-6	170.66	20.53	2.10	170.66	20.58[d]	2.10	
							F-1	170.08	20.63	2.12	170.09	20.69[d]	2.12	
1''	129.35(s)	-				132.20	F-3	169.63	20.53	2.17	169.65	20.56	2.18	
2'',6''	129.72(d)	7.49(d,8.8)				129.30	F-4	169.86	20.63	2.11	169.88	20.72	2.11	
3'',5''	117.10(d)	7.00(d,8.8)				121.90	F-6	170.46	20.63	2.12	170.46	20.63[d]	2.12	
4''	158.36(s)	-				152.00								
7''	144.80(d)	7.67(d,16.0)				143.80								
8''	116.32(d)	6.39(d,16.0)				118.00								
9''	165.64(s)	-				165.80								
1'''	98.47(d)	5.15(d,7.5)												
2'''	71.10(d)	5.30(m)												
3'''	72.59(d)	5.30(m)												
4'''	68.19(d)	5.18(dd,10.0,9.4)												
5'''	72.18(d)	3.90(ddd,10.0,5.2,2.5)												
6'''a	61.88(t)	4.30(dd,12.3,5.2)												
6'''b	-	4.18(dd,12.3,2.5)												
OMe	51.61(q)	3.75(s)			51.59(q)	3.75(s)								
OAc[b]	C=O	CH_3			C=O	CH_3								
	170.44	20.66	2.08		169.99	21.00	2.10							
	170.40	20.66	2.07		169.86	20.53	2.07							
	170.09	20.55	2.06		169.63	20.53	2.03							
	170.09	20.55	2.05		169.30	20.53	2.00							
	169.28	20.55	2.04		168.88	20.16	1.92							
	169.20	20.55	2.03											
	169.15	20.55	2.00											
	168.70	20.16	1.92											

a. $(OAc)^{13}$: δ1.911, 1.901, 1.896, 1.813, 1.792, 1.777, 1.762, 1.760, 1.734, 1.716, 1.715, 1.708, 1.651 (all singlet);
b. Chemical shifts described in decreasing order without correlation;
c. Assignments deduced by comparison with sucrose octaacetate;
d. Assignment may be interchanged.

greatly facilitated by comparison with the data for **7a**, the remaining absorptions were correlated with **9a** (Table 1) by comparative analysis with literature data described for peracetylated sucrose[10,11]. The solvent (benzene-d_6) effect was exploited to recognize clearly the signals of the thirteen acetoxyl groups (five in the molecule **8a** and eight in the **9a**) well resolved in the ^1H NMR spectrum. Further, the data obtained by ^1H NMR spectra permitted to deduce that the mixture contained approximately 50% of each component (**8a** + **9a**).

Finally, the presence of an isomer (~ 20%) of **7a** containing the *cis*-coumarate moiety was recognized mainly by signals at δ 7.60 (d, J=8.7 Hz, H-2",6"), 6.93 (d, J=8,7 Hz, H-3",5"), 6.92 (d, J=1.6 Hz, H-10), 5.91 (d, J=11.6 Hz, H-8"), 5.75 (qd, J=6.7 and 1.2 Hz, H-13), 3.12 (dd, J=8.6 and 2.5 Hz, H-9) and 1.54 (d, J=6.7 Hz, CH_3-13).

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