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Wood residues from *Zygia racemosa* (Ducke) Barneby & J.W. Grimes: Secondary metabolites, physical properties and anatomical aspects of the wood

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Abstract

Zygia racemosa (Ducke) Barneby & J.W. Grimes (syn. Pithecellobium racemosum Ducke) is a large tree with its geographic distribution restricted to South America (Brazil, Colombia, Guyana, French Guiana, Peru and Suriname). It has a wide occurrence in the Brazilian Amazon and is abundant in campinarana and terra firme forest. In the present work, we evaluated the secondary metabolites, physical properties and anatomical aspects of wood residues of the species. The chromatographic fractionations of the hexane extract led to the isolation of steroids identified as spinastenone (1) and spinaterol (2). The methanol extract provided the steroids 1 and nonanoate-cholest-7, 22-dien-3β-ol (3), the triterpenes oleanolic acid (4) and 3β,21β-dihydroxyolean-12-en-28-oic acid (5). The basic density found was 0.81 g/cm3 and the anisotropic factor was 1.90, which confirms its excellent quality. The acquisition of a sample of wood residues from Zygia racemosa was an opportunity to generate knowledge regarding the secondary metabolism, the physical properties and the anatomical aspects of the wood of this species. The steroids and triterpenes identified suggest that they are associated with plant defense in Z. racemosa.

Keywords: Fabaceae; angelin-rajado; triterpenes; steroids.

1. Introduction

Zygia P. Browne is a Neotropical genus that belongs to the Fabaceae-Mimosoideae family, and has about 114 species distributed from Central Mexico to Argentina with greater occurrence in the Peruvian Amazon. In Brazil, 18 species of this genus have been recognized, most of which occur in the Brazilian Amazon (Rico-Arce, 1994; Barneby and Grimes, 1997; Tropicos, 2021). Few secondary metabolites have been identified in the genus whose chemical skeletons are triterpenes, steroids and coumarins. Two triterpene saponins were found in the trunk bark of the Amazonian specimen *Zygia racemosa* (Ducke) Barneby & J.W. Grimes (syn. *Pithecellobium racemosum* Ducke) (Khan et al., 1997). A mixture of steroids (β-sitosterol, stigmasterol and campesterol) widely found in plants have been identified in the wood and trunk bark of *Zygia dulcis* (Roxb.) A. Lyons [syn. *Pithecellobium dulce* (Roxb.) Benth.] (Katekhaye and Laddha,

2015; Katekhaye et al., 2016). In the trunk bark of this species, two coumarins and two triterpenes were also identified (Katekhaye and Laddha, 2015; Katekhaye et al., 2016). One sterolglycoside and two sterolglycolipids were found in the branches of *Zygia cauliflora* (Willd.) Killip (*Pithecellobium cauliflorum* Mart.) (Gomes and Alegrio, 1998). Nine triterpene saponins were found in seeds of *Z. dulcis* (Barrera-Necha et al., 2003; Yoshikawa et al., 1997; Sahu and Mahato, 1994; Saxena and Singhal, 1998; Nigam et al., 1997).

Terpenes are the largest and most diverse class of secondary metabolites found in nature (Ashour et al., 2010; Dewick, 2002) and their production by plants is to counter biotic (pathogenic microbes, herbivorous pests and weeds) and abiotic stresses (water, temperature, light and salt) has been widely studied (Thimmappa et al., 2014; Silva et al., 2016, Mahdavi, et al., 2020). Triterpenes are formed from the cyclization of 2,3-oxidosqualene and are also precursors of steroids. Triterpenes and steroids have a wide occurrence in leaves and bark of Fabaceae. However, there are few studies regarding the wood in this family and little is known about the levels of these compounds in this part of the tree.

The species selected for this study, *Zygia racemosa,* is a large tree with geographic distribution restricted to South America (Brazil, Colombia, Guyana, French Guiana, Peru and Suriname) (Barneby & Grimes 1997). It has a wide occurrence in the Brazilian Amazon (Amapá, Amazonas, Maranhão, Mato Grosso, Pará and Rondônia states) and is abundant in campinarana and *terra firme* forest (REFLORA, 2021). In the present work, we evaluated the secondary metabolites, physical properties and anatomical aspects of wood residues of *Z. racemosa*.

2. Materials and Methods

2.1 General

NMR spectra were measured using a spectrometer (Bruker Fourier-300); chemical shifts (δ) were expressed in ppm, and coupling constants (*J*) in Hertz. Low-resolution ESI-MS was recorded using an ion trap mass spectrometer (Thermo Scientific LCQ Fleet). Column chromatography (CC) was performed with silica gel 60 (230-400 mesh), Analytical TLC was performed with silica gel 60 F_{254} (0.25 mm) pre-coated alumina sheets (Merck) visualized using UV light (254 and 365 nm), and vanillin-sulfuric acid spray.

2.2 Obtaining wood residues, identification and organic extracts

Samples of wood residues were classified and identified via macroscopic comparisons with standard samples of the species that were obtained from the xylotheque at the National Institute for Amazonian Research (INPA). Larger wood residues underwent previous evaluation of their technological properties and were used for experiments in the production of artifacts by the Wood Artifacts Engineering Laboratory at INPA. The smaller residues resulting from these procedures were made available for phytochemical studies. Extractions were performed by macerating the samples with hexane followed by methanol for a period of 7 days for each solvent at room temperature.

2.3 Macroscopic identification, determination of basic density and dimensional stability

The determination of the density (ratio between the oven-dried wood sample's mass and its saturated state volume) was conducted using 2 x 2 x 3 cm wood blocks. Their tangential and radial contractions were determined by direct mensuration with the aid of digital calipers. In addition, each wood sample's anisotropy coefficient (relation between the tangential and radial contractions) was calculated. In the macroscopic identification, the organoleptic and anatomical characteristics were analyzed. The analyses of the anatomical characteristics were carried out using a sample block that was observed with the naked eye and with the aid of a 10x magnifying glass.

2.4 Chromatographic fractionation of extract from *Zygia racemosa*

The hexane extract (0.61 g) was fractionated over silica gel in the column (230-400 mesh; h X Φ = 42 X 2.0 cm), eluted with hexane, hex:EtOAc (2-50%), to yield thirty-two fractions. Fraction 13 provided compound **1** (2.2 mg). Fraction 22 was fractionated over silica gel in the column (230-400 mesh; h X Φ = 37 X 1.5 cm), eluted with CH_2Cl_2:AcOEt (95:5), and resulted in 10 sub-fractions of which sub-fraction 3 provided compound **2** (24.6 mg). Fractionation of the methanol extract (4.44 g) was carried out in a silica gel column (230-400 mesh; h X Φ = 35 X 2.5 cm), eluted with hexane, hex:EtOAc (2-50%), EtOAc, EtOAc:MeOH (10-20%), and resulted in fifty eight fractions. Fractions 9, 16-19 and 40 provided coumpouds **1** (14.4 mg), **3** Pr (116.3 mg) and **4** (111.0 mg), respectively. Fractionation of the grouped fractions 44-45 over silica gel in the column (230-400 mesh; h X Φ = 30 X 1.2 cm), eluted with CH_2Cl_2, CH_2Cl_2:EtOAc (2-30%), resulted in the purification of compound **5** (6.1 mg).

2.5 Spectroscopic data of compounds

Spinastenone (**1**). Amorphous solid. ^{1}H NMR (300 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): Characteristic signals, 5.20 (1H, dd, J = 15.2, 8.7, H-22), 5.19 (1H, t, 5.3, H-7), 5.07 (1H, dd, J = 15.2, 8.8, H-23), 1.02 (3H, sl, H-19 and H-21), 0.86 (3H, d, 6.4, H-27), 0.84 (3H, d, 6.2, H-26), 0.84 (3H, t, 7.3, H-29), 0.58 (3H, s, H-18). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): Table 1.

Spinasterol (**2**). White solid. ^{1}H NMR (300 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): Characteristic signals, 5.19 (1H, dd, J = 15.1, 8.6, H-22), 5.16 (1H, m, H-7), 5.06 (1H, dd, J = 15.1, 8.5, H-23), 3.59 (1H, m, H-3), 1.03 (3H H, d, J = 6.6, H-21), 0.85 (3H, d, J = 6.4, H-26), 0.82 (1H, d, J = 5.7, H-27), 0.80 (3H, t, J = 7.3, H-29), 0.81 (3H, s, H-19), 0.55 (3H, s, H-18). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): Table 1. HRMS *m/z* 413.3683 $[M+H]^+$.

Nonanoate-cholest-7, 22-dien-3β-ol (**3**). Amorphous solid. ^{1}H NMR (300 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): Characteristic signals, 5.19 (1H, dd, J = 15.1, 8.5, H-22), 5.16 (1H, m, H-7), 5.06 (1H, dd, J = 15.1, 8.5, H-23), 3.66 (1H, m, H-3), 1.04 (3H, d, J = 6.7, H-21), 0.88 (3H, t, J = 6.5, H-29), 0.81 (6H, d, J = 6.4, H-26 and H-27), 0.80 (1H, s, H-19), 0.55 (1H, s, H-18). Side chain: 2.35 (2H, t, J = 7.4, $\underline{CH_2}$-CO), 1.60 (4H, m, $\underline{CH_2}CH_2CH_3$), 1.25 $(CH_2)n$, 0.86 (3H, m, $\underline{H_3C}CH_2n$). ESIMS *m/z* 553.49 $([M+1]^+$.

Oleanolic acid (**4**). White solid. ^{1}H NMR (300 MHz, TMS, C_5D_5N, δ, ppm, J/Hz): Characteristic signals, 5.48 (1H, t, J = 3.2, H-12), 3.45 (1H, tl, H-3), 1.25 (3H, s, H-27), 1.21 (3H, s, H-23), 1.00 (9H, s, H-24, H-

26 and H-30), 0.92 (3H, s, H-29), 0.86 (3H, s, H-25). ^{13}C NMR (75 MHz, C_5D_5N,, δ, ppm): Table 1. HRMS *m/z* 457.3658 [M+H]+.

3β,21β-Dihydroxyolean-12-en-28-oic acid (**5**). White solid. ^{1}H NMR (300 MHz, TMS, $(CD_3)_2CO$, δ, ppm, J/Hz): Characteristic signals, 5.31 (1H, t, J =3.6, H-12), 4.56 (1H, sl, H-21), 3.18 (1H, dd, J =10.0, 5.3, H-3), 1.42 (3H, s, H-23), 0.98 (6H, s, H-29 and H-30), 0.94 (3H, s, H-25), 0.88 (3H, s, H-23), 0.78 (6H, s, H-24 and H-26).

3. Results and Discussion

The sample of the species identified as *Zygia racemosa* (Ducke) Barneby & J.W. Grimes presented the following organoleptic characteristics: light yellow to orange-brown color, covered by dark brown stretch marks that confer a distinct beauty. The wood has a low to medium shine, and has no odor or taste. The basic density found was 0.81 g/cm^3, which is considered a high density. It is a very heavy and hard wood, and is difficult to cut with hand tools. The anisotropic factor found was 1.90 (Table 1), which confirms its excellent quality since, the higher the anisotropy coefficient, the lower the mechanical resistance to drying defects such as warping of the wood. Figure 1 shows the macroscopic characteristics of the wood that presents a paratracheal axial parenchyma, predominantly vasicentric, aliform, with short and oblique confluences, occasionally with thin terminal lines. Radial parenchyma in irregular arrangement. Small to medium pores, predominantly solitary, multiples of 2-3, clogged by wood-colored resin.

Table 1. Physical properties of *Zygia racemosa*

Basic density (g/cm^3	Tangential contraction (%)	Radial contraction (%)	Anisotropic factor
0.81	12.4	6.54	1.90

Figure 1. Macrography of the transversal plane (10x magnification) of *Zygia racemosa*

The identification of compounds (Figure 2) was based on magnetic nuclear resonance data, (one and two-dimensional), mass spectrometry and comparisons with the literature.

The ^{1}H NMR spectra of **1** and **2** showed signals that are characteristic of steroids due to the shifts of six methyl groups (δ 1.03-0.55) and olefinic hydrogens at δ 5.19 (t, J = 5.3, H-7), 5.20 (dd, J = 15.2, 8.7, H-22), 5.07 (dd, J = 15.2, 8.8, H-23) of **1**; δ 5.16 (m, H-7), 5.19 (dd, J = 15.1, 8.6, H-22), 5.06 (dd , J = 15.1, 8.5, H-23) of **2**. The spectral difference in **2** was the appearance of the multiplet at δ 3.65, which is characteristic of a 3-position oxymethinic hydrogen and the heteronuclear single quantum correlation (HSQC) experiment showed correlation of this hydrogen with the carbon at δ 71.1. Position 3 of compound **1** has the carbonyl signal at δ 212.1 (Table 1). The ^{1}H and ^{13}C NMR data of **2** were similar to those published by Segrero et al. (2017). The steroids were identified as spinastenone and spinastenol, respectively. In the Fabaceae family, spinasterol has been reported in several species of the subfamily Mimosaceae; however, in wood, it has only been registered in the species *Pithecellobium multiflorum* (Gunasekera et al., 1982) and *Acacia mangium* (Melo et al., 2020).

The ^{1}H NMR spectrum of **3** also showed characteristic steroid signals similar to **2**. The ^{13}C NMR spectrum (Table 1) showed a carbonyl signal at δ 179.3, suggesting an ester in the side chain at position 3 of the molecule. The heteronuclear multiple bond correlation (HMBC) experiment showed correlations of the hydrogen side chain at δ 2.35 (H-2'), with the carbons at δ 179.3 (C-1'), 29.7 (C-n') and 24.7 (C3'). ESI-MS indicated the molecular formula $C_{38}H_{64}O_2$, the molecular ion peak at *m/z* 553 ([M+1]+) and the fragment at *m/z* 413, indicates the loss of $C_9H_{17}O$, which refers to the spinasterol derivative side chain. Thus,

compound 3 was identified as nonanoate-cholest-7, 22-dien-3β-ol that is present in the methanol extract of *Z. racemosa* at the percentage of 2.6%.

The ^{1}H NMR spectra of **4** and **5** showed characteristic signals of oleanane triterpene due to the double olefin triplet observed at δ 5.48 (t, 3.2 Hz) of compound **4** and δ 5.31 (t, 3.6 Hz) of **5**, oxymethinic hydrogens in position 3 at δ 3.45 (tl, compound **4**) and δ 3.18 (dd, compound **5**), in addition to the presence of seven methyls each in the region of δ 1.21-0.86. The ^{1}H NMR spectrum of **5** showed a broad singlet at δ 4.56, which is characteristic of oxymethinic hydrogen, and indicative of an additional hydroxyl in the molecule. The ^{13}C NMR spectra showed chemical shifts of the acid carbonyls observed at 179.9 and 177.6 (Table 1), respectively. The ^{13}C NMR data of **4** were similar to those published by Seebacher et al. (3003) for oleanolic acid. The HMBC of **4** showed correlations of the hydrogens at δ 3.29 (H-18) with the signals of carbons at δ 179.9 (C-28), 144.6 (C-13), 122.3 (C-12), 46.4 (C-17) and 41.9 (C-14) that confirmed the position of the acid at C-17. The HMBC of **5** showed the correlations of the signals at δ 3.09 (H-18), 1.91 (H-22) and the methyls δ 0.98 (H-29, 30) with the carbon signal at δ 74.0, confirming the oxygenation in C-21. Thus, compound **5** was identified as 3β,21β-dihydroxy-olean-12-en-28-oic acid, whose 13C NMR data were compared with its glycosylated derivative published by Mimaki et al. (2004).

Oleanolic acid is a triterpenoid of the oleanane skeleton with widespread occurrence in wood or herbaceous plants of several families. The biological roles of oleanolic acid are often associated with the formation of a barrier against water loss and pathogens (Heinzen et al., 1996). Allelopathic properties have also been described for this compound (Szakiel et al., 2003). In this study of wood residues of *Zygia racemosa,* oleanolic acid was found in a high percentage (2.5%).

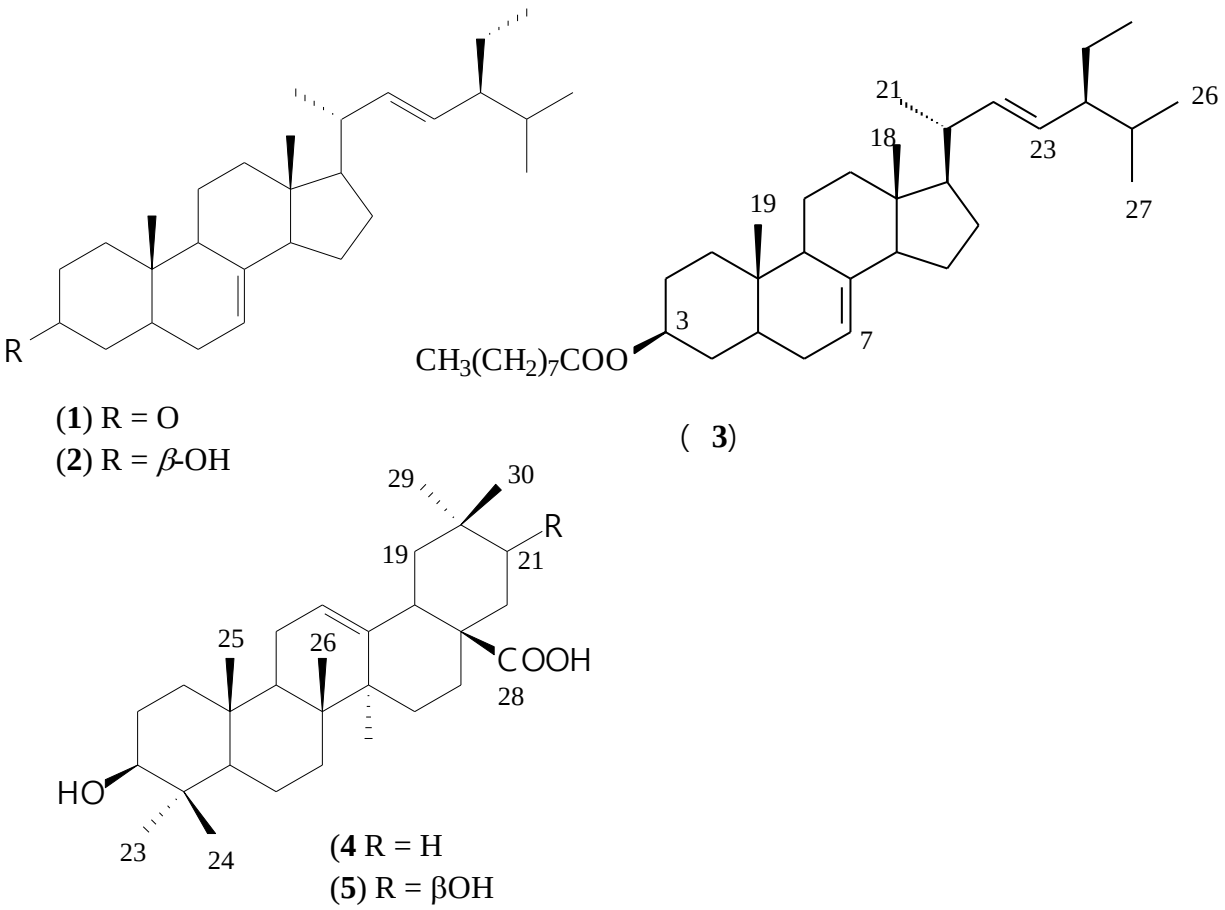

Figure 2. Triterpenes and steroids from wood residues of *Zygia racemosa*

Table 1. RMN ^{13}C data for steroids **1**, **2** ($CDCl_3$) and triterpenes **4** (C_5D_5N), **5** $(CD_3)_2CO$.

N0	1	2	3	4	5
1	38.8	37.1	37.1	38.7	38.5
2	38.1	31.4	31.4	27.6	27.2
3	212.1	71.0	71.0	77.8	77.7
4	44.3	37.9	37.9	39.2	38.6
5	42.9	40.2	40.2	55.5	55.4
6	30.1	29.6	29.6	18.5	18.2
7	117.0	117.4	117.4	33.9	33.0
8	139.5	139.5	139.5	39.5	39.3
9	48.8	49.4	49.4	47.9	46.7
10	34.4	34.2	34.2	37.1	36.9
11	21.7	21.5	21.5	23.4	23.4
12	39.3	39.4	39.4	122.3	122.0
13	43.3	43.2	43.2	144.6	144.0
14	55.0	55.1	55.1	41.9	41.32
15	23.0	23.0	23.0	28.1	30.25
16	29.7	28.5	28.5	23.4	31.80

17	55.9	55.8	55.8	46.4	47.81
18	12.1	12.0	12.0	41.8	40.6
19	12.3	13.0	13.0	46.2	46.5
20	40.8	40.8	40.8	30.7	35.4
21	21.4	21.3	21.3	32.9	74.0
22	138.1	138.1	138.1	30.7	35.1
23	129.5	129.4	129.4	28.5	32.5
24	51.2	51.2	51.2	16.3	15.5
25	31.9	31.8	31.8	15.3	15.0
26	19.0	21.1	21.1	17.2	16.7
27	21.1	18.9	18.9	25.9	26.3
28	25.4	25.4	25.4	179.9	177.6
29	12.5	12.2	14.1	33.0	27.8
30				23.5	23.8
1’			179.2		
2’			33.9		
3’			24.7		
4’ – 6’			29.7-29.0		
7’			21.1		
8’			22.7		
9’			13.09		

4. Conclusion

The acquisition of a sample of wood residues from *Zygia racemosa* was an opportunity to generate knowledge regarding the secondary metabolism, physical properties and anatomical aspects of the wood of this species. The phytochemical study led to the isolation and identification of steroids and triterpenes that suggest that they are associated with plant defense because, in general, triterpenoids (including steroids) are widely distributed in plants and are considered defense compounds against pathogenic microbes and herbivores.

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