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Original Article

Study in wood of *Ceiba pentandra* (L.) Gaertn. from planting: chemical constituents and their biological importance

Estudo em madeira de *Ceiba pentandra* (L.) Gaertn. proveniente de plantio: constituintes químicos e sua importância biológica

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Abstract

Ceiba pentandra (Malvaceae) is a species popularly known in North Brazil as "sumaúma" that has attracted interest in phytochemical studies due to the diversity of classes of compounds biologically active. The objective of this study was to investigate the chemical constituents present in the wood residues of *Ceiba pentandra* and to evaluate the antibacterial activity of a purified sesquiterpene lactone against *Xanthomonas citri* subsp. *citri*. The isolated compounds were identified based on one-dimensional (^{1}H and ^{13}C) and/or two-dimensional (HSQC and HMBC) NMR analyses, the mixtures of fatty acid were characterized by GC-MS. The chemical constituents identified were steroids (cholest-4-en-3-one, stigmast-4-ene-3,6-dione, β-sitosterol, and stigmasterol); macrolides (des-*O*-methyl-lasiodiplodine and lasiodiplodine); isocoumarin (4-hydroxymelein); sesquiterpene lactone (isohemigosilicic acid lactone-2-methyl ether); and a mixture of fatty acids (palmitic acid, oleic acid, stearic acid, linoleic acid, and dihydrosterculic acid). The sesquiterpene lactone was evaluated against the bacterium *Xanthomonas citri* ssp. *citri* and demonstrated bactericidal and bacteriostatic activities at concentrations up to 400 μg mL^{-1}. Our phytochemical study aggregated knowledge of this wood species originated from plantation sites and found activity bactericide to combat citrus canker.

Keywords: macrolides, fatty acid, NMR, citrus canker, Malvaceae.

Resumo

Ceiba pentandra (Malvaceae), popularmente conhecida no Norte do Brasil como "sumaúma" tem despertado interesse nos estudos fitoquímicos devido à diversidade de classes de compostos biologicamente ativos. O objetivo desse estudo foi investigar os constituintes químicos dos resíduos madeireiros de *C. pentandra* e avaliar a atividade antibacteriana de uma lactona sesquiterpênica purificada contra *Xanthomonas citri* subsp. *citri*. Os compostos isolados foram identificados com base nas análises de RMN unidimensional (^{1}H e ^{13}C) e/ou bidimensional (HSQC e HMBC), as misturas de ácidos graxos foram caracterizadas por GC-EM. Os constituintes químicos identificados foram os esteroides (colest-4-en-3-ona, estigmast-4-eno-3,6-diona, β-sitosterol e estigmasterol); macrolídeos (des-*O*-metil-lasiodiplodina e lasiodiplodina); isocumarina (4-hidroximeleína); lactona sesquiterpênica (ácido isohemigossílico lactona-2-metil éter); mistura de ácidos graxos (ácido palmítico, ácido oleico, ácido esteárico, ácido linoleico e dihidroestercúlico). O sesquiterpeno lactona foi avaliado frente a bactéria *Xanthomonas citri* ssp. *citri* e demostrou atividades bactericida e bacteriostática em concentrações de até 400 μg.mL^{-1}. Nosso estudo fitoquímico agregou conhecimento sobre essa espécie de madeira originária de áreas de plantio e encontrou atividade bactericida no combate ao cancro cítrico.

Palavras-chave: macrolídeos, ácido graxo, RMN, cancro cítrico, Malvaceae.

1. Introduction

The species *Ceiba pentandra* (L.) Gaertn., belonging to the Malvaceae family, is known in the Amazon region as "sumaúma" or "samaúma," where it is called by indigenous peoples the "mother of trees" these trees reach dimensions close to 60 meters in height and 3 meters in diameter, making it one of the largest trees in Brazil (Carvalho, 2008).

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Presents basais tubulares roots known as "sapopemas" (from Tupi, meaning "flat root"), which are used as shelters by indigenous and riverine dwellers of the Amazon region (Souza et al., 2005). The roots produce sounds that resonate over long distances when touched like this, indigenous peoples use these touches to communicate with each other (Cantalice et al., 2020). It is considered a fast-growing tree with reproduction occurring mainly by cross-pollination. One of its propagation methods is seed dispersal by wind. This occurs when the fruit capsule falls to the ground and ruptures, releasing a mass of cotton-like fiber (known as "kapok") that contains the seeds (Gribel et al., 1999).

The wood of "sumaúma" has a low basic density (0.32 to 0.34 g.cm^{-3}) (Freitas and Vasconcellos, 2010) and is widely used in the production of plywood panels in the Brazilian Amazon, with high commercial value. In the Amazon, the species' habitat is terra firme forest and flooded areas and has been identified as having potential for planting in region Amazon, especially in agroforestry systems. Souza et al., (2010) evaluated native and exotic forest species in *terra firme* under full-sun planting conditions and found that C. pentandra stood out among the forest species with the best results in growth (height and diameter) and volume production indicating its high potential for timber purposes under these conditions, thus being considered one of the promising native species for commercial planting in the region.

Phytochemical studies reported on native specimens of *C. pentandra* demonstrate the diversity of secondary metabolites in different vegetative parts, such as fatty acids of seeds, fruits and leaves (Kaimal and Lakshminarayana, 1970; Kiran and Rao, 2014; Abouelela et al., 2018), steroids of bark, stem bark and leaves (Nelly et al., 2025; Onuh et al., 2024; Abouelela et al., 2018), triterpenes of bark and leaves (Nelly et al., 2025; Abouelela et al., 2018), sesquiterpenes of root bark and heartwood (Rao et al., 1993; Kishore et al., 2003), xanthones of inflorescences (Taiwo et al., 2024), flavonoids of leaves and stem bark (Aderogba et al., 2013; Onuh et al., 2024; Ngounou et al., 2000; Ueda et al., 2002; Nelly et al., 2025; Noreen et al., 1998), flavanolignans of aerial parts (Abouelela et al., 2020), as well as phenolic acids of bark stem and leaves (Nelly et al., 2025; Onuh et al., 2024; Aderogba et al., 2013).

In this paper, we present the results of a phytochemical study on *C. pentandra* wood from planting and antibacterial activity against *Xanthomonas citri* subsp. *citri*, the etiological agent of citrus canker.

2. Material and Methods

2.1. General methods

Nuclear Magnetic Resonance (NMR) was measured using spectrometers (Bruker Fourier-300; Bruker Avance- 400 MHz; Bruker Avance IIIHD- 500 MHz); chemical shifts (δ) were expressed in ppm and coupling constants (*J*) in Hertz. Gas chromatograph (GC) (Nexis GC2030, GCMS-QP2020 NX, Shimadzu). Column chromatography (CC) was performed with a silica gel 60 (230-400 mesh (Sigma-Aldrich, Gallen Switzerland) and Sephadex LH-20 (Sigma-Aldrich, Gallen Switzerland). Analytical TLC was performed with silica gel 60 F_{254} (0.25 mm) pre-coated aluminum sheets (Merck, Darmstadt, Germany) visualized using UV light (254 and 365 nm) and vanillin-sulfuric acid spray. Deuterium solvent (Merck, Darmstadt, Germany). Easydry-type microwave oven (Wu M. Gobler, capacity 0.7 m^3).

2.2. Acquisition of woody residues, identification and drying

Ceiba pentandra was planted in 1992 at the Embrapa experimental research station in Manaus, Amazonas. The plantation was part of an experiment called "full-sun forest species trial in a *terra firme* ecosystem," which included 13 other species. The seedlings were planted at 3x3 m spacing, in four plots of 25 plants each. Wood samples from a specimen of plantation about 25 years old (SISGEN number A42EE82) were obtained from Embrapa Ocidental (2°53'36.3" S 59°58'23.0" W) and identified by comparison with samples available in the xylotheque of the National Institute for Amazonian Research (INPA). The wood residues were subjected to drying in a microwave oven of the Easydry type (Wu M. Gobler), with a capacity of 0.7 m^3.

2.3. Extraction and isolation

The samples (1.2 kg) of *Ceiba pentandra* were ground and subjected to extraction with hexane and then with methanol for a period of 7 days at room temperature. The methanol extract (3.02 g) was fractionated over silica gel in a column (230-400 mesh; h (height) × Ø (diameter) = 25 X 2.5 cm) and eluted with hexane, hex:EtOAc and EtOAc yielding thirty-two fractions. Fraction 11 was fractionated over silica gel in the column (230-400 mesh; h X Φ = 21.5 X 0.6 cm) and eluted with CH_2Cl_2 resulting in the purification of compound **1** (2.3 mg). Fractions 12 (14.6 mg), 14 (23 mg), 16 (128 mg), 17 (94.2 mg), 18 (52 mg) and 21 (39.6 mg) were subjected to complementary chromatographic procedures. Fr. 12 was subjected to silica gel in column chromatography (230-400 mesh; h X Φ =15.2 X 0.6 cm), eluted with hex:EtOAc (7:3) to give compound **2** (4 mg). Fr. 14 was recrystallized in cold acetone to give compounds **3a** and **3b** (2.2 mg), and then the mother liquor, after drying, was chromatographed over a silica gel column (230-400 mesh; h X Φ =30 X 1.5 cm), eluted with CH_2Cl_2: EtOAc (98:2), which resulted in 15 sub-fractions. Sub-fraction 5 (5.4 mg) was fractionated over silica gel in a column (230-400 mesh; h X Φ =6.5 X 0.4 cm), eluted with CH_2Cl_2:EtOAc (98:2) to give compound **4** (3.5 mg). Fractions 15 to 22 (422.8 mg) showed a predominance of fatty acids in their composition, however, only fractions 16, 17, 18, and 21 were subjected to purification. Fr. 16 was chromatographed over Sephadex LH-20 (h X Φ =38.5 X 2.4 cm) and eluted with MeOH to give a mixture of fatty acids **5** (89.3 mg). Fr. 17 was fractionated over silica gel in a column (230-400 mesh; h X Φ = 38 X 1.8 cm) and eluted with CH_2Cl_2 resulting in 35 sub-fractions. Sub-fraction 11 provided the mixture of fatty acids **6** (50.4 mg) and sub-fraction 17 was recrystallized in cold hexane to give compound **7** (2.6 mg). Fractionation of fraction 18 over silica gel in the column (230-400 mesh; h X Φ = 38 X 1.3 cm), eluted with CH_2Cl_2:MeOH (98:2)

resulted in the purification of compound **8** (3.6 mg). Fr. 21 was fractionated over silica gel in a column (230-400 mesh; h X Φ = 36 X 1.2 cm), eluted with CH_2Cl_2: EtOAc (98:2) to give compound **9** (1.5 mg).

2.4. Gas chromatography-mass spectrometry analyses

The analyses of the mixture of fatty acids were performed at a concentration of 1 mg/mL in hexane with a GC equipped with a split-splitless injector and an auto sampler. The fatty acid methyl esters (FAMEs) were separated using a fused silica capillary column SHRTx-5Sil-MS (30 m length, 0.25 mm internal diameter and 0.25 µm film thickness). Helium was used as carrier gas at 2 mL/min. Injection temperature was 260 °C, split mode, 1 µL, and the mass spectrometer ion source and interface temperatures were 230 and 280 °C, respectively. The chromatographic analysis started at 50 °C and increased at 20 °C/min until reaching 210 °C, where it remained for 18 min, and then increased to 230 °C at 20 °C/min and was maintained for 13 min. The total running time was 40 min. The mass spectra were obtained by electronic impact at 70 eV. Identification of the fatty acids was via the WILEY 275 and National Institute of Standards and Technology (NIST 3.0) databases.

The fatty acid profile samples were analyzed after derivatization to FAMEs. Thus, 20 mg of samples were mixed with 0.5 mL of chloroform:methanol (2:1; v/v) and 1 mL of 0.1 M NaOH in methanol. This mixture was heated to 60 °C for 30 min and then the reaction was stopped with the addition of 0.2 mL of distilled water, and the FAMEs formed were extracted with the addition of 1 mL of hexane. After stirring, the mixture was decanted and the isolated upper phase was collected, according to the methodology of Vasquez et al. (2021) with modifications. The procedure was repeated with the lower phase to recover the remaining FAMEs, and was shaken and left to stand for 30 min.

2.5. In vitro antibacterial assay

The antibacterial activity of compound **8** was measured by a slight modification of the of the microplate microdilution method. Compounds and positive control (copper sulfate) and dissolved in DMSO and then diluted with nutrient broth (NB). A bacterial suspension of 5 x 10^5 CFU was prepared and inoculated into the 96-well plate. Serial dilutions were prepared in each well to make the final concentrations of 2000 a 0.98 µg/Ml. The plates were then incubated at 28 ºC for 72 h then revealed with a resazurin aqueous solution (30 µL; 0.02% w/v, Sigma Aldrich, St. Louis, MO, USA) to determine the minimum inhibitory concentration (MIC) of each compound. The experiments were performed in triplicate. To determine the Minimum Bactericidal Concentration (MBC), 10 µL of each well taken from the CIM assay was cultured on the surface of nutrient agar (NA) medium and incubated for 72 h at 28 ºC. The complete absence of bacterial growth was considered the MBC. Resazurin solution was used in an indicator. The experiments were performed in triplicate.

3. Results and Discussion

The chromatographic fractionations of the methanolic extract of the wood residues from *C. pentandra* resulted in the identification of steroids (**1**, **2**, **3a** and **3b**), macrolides (**4** and **9**), fatty acid (**5a**, **5b**, **5c**, **6a** and **6b**), an isocoumarin (**7**) and a sesquiterpene lactone (**8**) (Figure 1). The structures of the isolated compounds were established based on

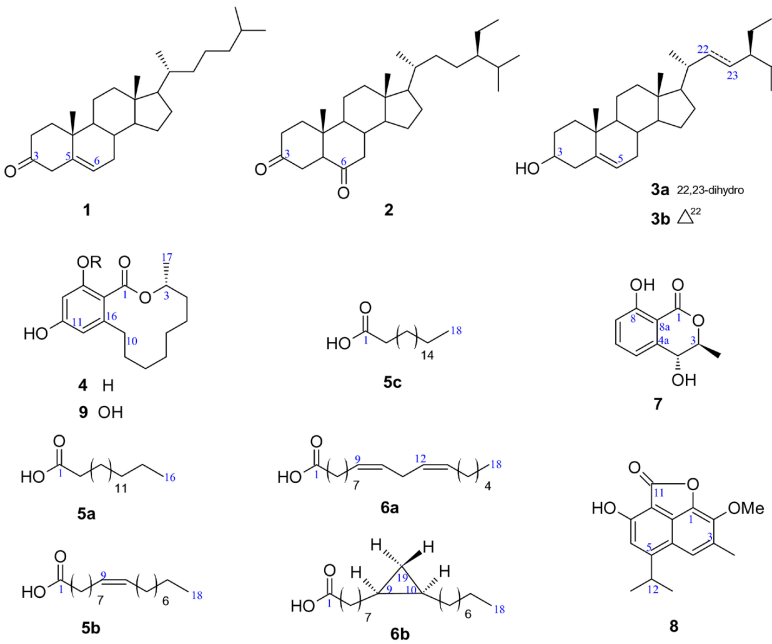

Figure 1. Compounds from wood of *Ceiba pentandra* (L.) Gaertn. from planting.

^{1}H and ^{13}C NMR spectra and comparison with literature data, the characterization of fatty acid methyl esters were characterized by GC and NMR.

The NMR spectra of compounds **1-3** showed characteristic steroid signals. The ^{13}C NMR spectrum of **1** showed the presence of a carbon–carbon double bond (δ 171.9 and 123.7) and indicated the presence of conjugated carbonyl (δ 199.8), in the compound **2** the presence of a carbon–carbon double bond was observed at δ 161.1 and 125.5, and two conjugated carbonyls at δ 199.5 and 202.4. Thus, the cholestane-type steroid was identified as cholest-4-en-3-one (**1**) and the stigmastane-type steroid was identified as stigmast-4-ene-3,6-dione (**2**), both identified for the first time in *C. pentandra*. A mixture of β-sitosterol (**3a**) and stigmasterol (**3b**) has been widely found in various plant species, including *C. pentandra* (Abouelela et al., 2018; Onuh et al., 2024).

The ^{1}H NMR spectra of **4** and **9** showed signals of aromatic *m*-coupled hydrogens at δ 6.20 (H-11) and 6.25 (H-13) of compound **4**, at δ 6.24 (H-11) and 6.22 (H-13) of **9**, as well as signals corresponding to long-chain methylenes (δ 3.26-1.42). ^{13}C NMR spectrum of **4** indicated the presence of aromatics, seven methylene, methyl and an ester carbonyl carbon (at 171.8). The spectral data of compound **9** were similar to those of **4** except for the presence of methoxy. Thus, based on the data of NMR spectra data in 1D (^{1}H, ^{13}C and DEPT) and 2D (HSQC and HMBC), these macrolides were identified as des-*O*-methyl-lasiodiplodin (**4**) and lasiodiplodin (**9**), previously identified in Malvaceae (Rudiyansyah and Garson, 2006), as well as in other families of the plant kingdom, such as Fabaceae (Sousa et al., 2013), Moraceae (Chen et al., 2010), Boraginaceae (Yao et al., 1991; Xin-Sheng et al., 1983; Li et al., 2012), Euphorbiaceae (Cambie et al., 1991), Gentianaceae (Magadula et al., 2008) and Simaroubaceae (Mulholland et al., 2003), but reported here for the first time in the genus *Ceiba*.

The mixture of fatty acids such as palmitic acid (**5a**), oleic acid (**5b**), stearic acid (**5c**), linoleic acid (**6a**), and dihydrosterculic acid (**6b**) based on GC and NMR analyses. Tables 1 and 2 show the main fatty acids identified via GC analysis in fraction 16 and 17). The methanolic extract showed a high concentration of fatty acids in fractions 16 and 17 (4.62%) with a predominance of palmitic acid (fr 16) and dihydrosterculic acid (fr. 17).

In the ^{1}H-NMR spectrum, the signal at δ 5.4–5.3 corresponds to olefinic hydrogens (–CH=CH–) of unsaturated fatty acids. The signal at δ −0.34 (*dd*) can be attributed to one *cis* hydrogen of the methylene moiety in the cyclopropane ring of dihydrosterculic acid (**6b**) while the *trans* hydrogen of the cyclopropane methylene unit was observed at δ 0.56. The ^{13}C NMR spectrum showed typical carbon signals at δ 10.9 ppm for the methylene carbon and at 15.8 and 15.7 ppm for the methine carbons of the cyclopropane ring. These NMR data are in agreement with the literature (Knothe, 2006). The fatty acids from *C. pentandra* were found mainly in the seeds, in which were identified saturated, mono- and polyunsaturated in addition to cyclopropane fatty acids such as malvalic acid and sterculic acid (Montcho et al., 2018; Mondal, 2015; Kiran and Rao, 2014). However, the presence of methyl dihydrosterculate had not been previously reported in this species.

In our study with planting specimens, the high level of fatty acids found in wood residues may be related to the age of the tree, approximately 25 years old. Fatty acids are energy sources used by trees and play an important role in plant–pathogen interactions. Levels of fatty acids increase in response to various stresses abiotic and biotic stress, leading to increased plant defense and adaptation (Walley et al., 2013). The biosynthesis of fatty acids has much in common with the biosynthesis of macrolides, as their chemical mechanisms involved in chain extension are similar and the necessary precursors are acetyl coenzyme A (CoA) and malonyl-CoA (MCoA) units. Biosynthesis occurs via the acetate pathway and the main difference between their formation is that in the macrolide mechanism the

Table 1. The main fatty acids of *C. pentandra* wood fraction 16.

Peak	Retention time (min)	Fatty acid	Area (%)
1	13.53	Palmitic acid (**5a**)	35.38
2	16.84	Oleic Acid (**5b**)	4.32
3	17.44	Stearic acid (**5c**)	2.38

Table 2. The main fatty acids of *C. pentandra* wood fraction 17.

Peak	Retention time (min)	Fatty acid	Area (%)
1	13.03	Palmitic acid (**5a**)	13.94
2	15.80	Linoleic acid (**6a**)	3.48
3	15.95	Oleic acid (**5b**)	13.88
4	16.52	Stearic acid (**5c**)	5.17
5	18.79	Methyl dihydrosterculate (**6b**)	24.97

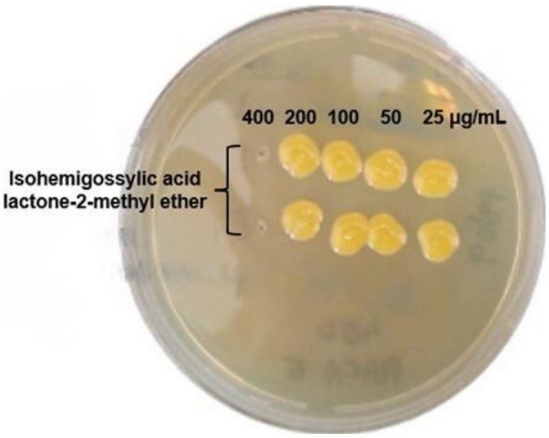

Figure 2. Microbial growth on nutrient agar medium.

reduction is variable, while the fatty acid product is reduced with each elongation of the chain (Hertweck, 2009).

The structure of compound **7** was established by 1D (^{1}H NMR, ^{13}C NMR) and 2D NMR (HSQC and HMBC) spectral analyses. The ^{1}H NMR spectrum showed signals corresponding to three aromatic hydrogen atoms at δ 7.60 (H-6), 7.13 (H-5), and 6.93 (H-7) and two oxymethine hydrogens. Analysis of the ^{13}C NMR spectrum revealed 10 signals, of which carbonyl group at δ 161.4, oxymethine carbons at δ 80.2 (C-3) and 68,2 (C-4), a methyl carbon at δ 17.3 (CH_3) and carbons of the aromatic system (δ 161.6, 143.4, 136.5, 116.4 and 107.0). Based on the literature (Asha et al., 2004), compound **7** was identified as *trans*-4-mellein, has previously been isolated from two species of the genus *Durio* in the family Malvaceae (Rudiyansyah and Garson, 2006).

The structure of the sesquiterpene **8** was established by 1D (^{1}H NMR, ^{13}C NMR) and 2D NMR (HSQC and HMBC) spectral analyses. The ^{1}H NMR spectrum displayed the presence of three methyl groups at δ 2.40 (3H, *s*, H-15) and δ 1.39 (6H, *d*, *J*= 6.8, H-13 and H-14), an isopropyl methine proton at δ 3.69 (*sept*, *J* = 6.8, H-12), a methoxy group at δ 4.31 (OCH_3), two aromatic hydrogens at δ 6.99 and 7.51 (H-6 and H-4). The ^{13}C NMR spectrum showed signals for 16 carbon atoms, including an ester carbonyl at δ 165.5 (C-11). Thus, the structure was identified as isohemigossylic acid lactone 2-methyl ether. The ^{1}H and ^{13}C NMR data are consistent with those reported by Puckhaber and Stipanovic (2001). This sesquiterpene lactone was previously isolated from the root bark of *C. pentandra* (Rao et al., 1993).

In this pioneering phytochemical study of a plantation specimen of *C. pentandra*, we identified steroids, macrolides, fatty acids (including those with cyclopropane rings), an isocoumarin, and a sesquiterpene lactone. A key finding of this study is the predominance of fatty acids across most of the methanol extract fractions.

Regarding the antibacterial activity, sesquiterpene lactone **8** exhibited activity against *Xanthomonas citri* subsp. *citri* (*Xcc*), with identical MIC and MBC values of 400 µg/mL, thereby demonstrating moderate bactericidal effects (Figure 2). The scarcity of literature reports on the antibacterial effects of isolated compounds against the *Xcc* bacterium supports the significance of the anti-*Xanthomonas citri* activity exhibited by the sesquiterpenes α-cadinol and spathulenol (Lima et al., 2025). These findings indicate the potential of this class of natural compounds as antimicrobial agents for the control of citrus canker.

4. Conclusion

Our phytochemical study on *Ceiba pentandra* (L.) wood has expanded the knowledge of this species from plantation sites. Molecules from different classes of secondary metabolites were identified, but the predominance of fatty acids which are uncommon in wood was evident. This suggests the importance of these compounds in defense and adaptation of the specimen.

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Data Availability Statement

All data supporting the findings of this study are available in the article and the Supplementary Material.

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Supplementary Material

Supplementary material accompanies this paper.
Physical and spectroscopic data of compounds 1-9
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