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Isolation and characterization of novel compounds from *Curcuma amada* Rhizomes: A phytochemical analysis

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Abstract
Curcuma amada Roxb., known as "Mango ginger," is a perennial aromatic herb of the Zingiberaceae family from the Indo-Malayan region. This plant is significant in traditional medicinal practices, such as Ayurveda and Unani. The rhizomes possess antibacterial, anti-inflammatory, antioxidant, anticancer, and antidiabetic properties. This study focused on isolating and identifying novel compounds from *C. amada* rhizomes using chromatographic and spectroscopic methods. Methanol extraction of rhizomes was followed by purification using column and thin-layer chromatography (TLC). Two novel compounds were isolated, and their structures were determined using High-Resolution Mass Spectrometry (HR-MS), Fourier-Transform Nuclear Magnetic Resonance (FT-NMR), and Fourier-Transform Infrared Spectroscopy (FT-IR). The first compound, 5, 8-dihydroxy-7-methyl-4-oxo-1-hydronaphthalen-1-yl 4'-ethyl-2', 5'-dimethylhexanoate, was isolated as a dark brown sticky substance with a molecular ion peak [M+] at 360.16 ($C_{21}H_{28}O_5$). The second compound, 8-dihydroxy-7-methyl-4-oxo-1-hydronaphthalen-1-yl 5'-hydroxy-2', 4', 5'-trimethylhexanoate, appeared as a dark brown sticky substance, with m/z 362.14 ($C_{20}H_{26}O_6$). The ^1H and ^{13}C NMR spectra provided structural analysis data. This study first isolated these compounds from *C. amada* Rhizomes, warranting further research into their biological activities.

Keywords: *Curcuma amada*, Zingiberaceae, rhizomes, isolation, characterisation, novel compounds

Introduction
Curcuma amada Roxb., a perennial aromatic herb with rhizomes, is a member of the Zingiberaceae family. Commonly referred to as "Amahaldi," "Amba ada," or "Mango ginger," its rhizome is distinguished by a flavor reminiscent of raw mango [1, 2]. Native to the Indo-Malayan region, this plant has disseminated to tropical regions across Asia, Africa, and Australia [3]. In India, it is cultivated in various states, including Odisha [2], although commercial propagation has not yet been documented. *C. amada* has been integral to traditional medicinal systems such as Ayurveda and Unani since ancient times, functioning as a coolant, appetizer, alexipharmic, antipyretic, aphrodisiac, diuretic, emollient, expectorant, and laxative. It is also utilized in the treatment of biliousness, itching, skin diseases, bronchitis, asthma, hiccups, and inflammation from injuries [4, 5]. The rhizome, when combined with common salt, is employed to treat colds and coughs and to enhance blood quality [6]. Furthermore, the rhizome facilitates the healing of wounds, cuts, and itching [7], while the leaves are applied to contusions and sprains [8, 9]. A paste made from the entire plant mixed with peppers is used for treating piles [6, 8]. *Curcuma amada* exhibits a spectrum of activities, including antibacterial, cytotoxic, and thrombolytic [11, 30], antiinflammatory [12, 19, 25, 28, 30], antimicrobial [13, 14, 16], antioxidant [10, 11, 14, 17, 30, 31], anticancer [14, 21, 27], analgesic [15, 31], anthelmintic [18, 32], antidiabetic [20, 30], antibacterial [11, 22], and antiobesity [23] properties, as well as anti-genotoxic [24], hepatoprotective [26], antifungal [29], antipyretic [33], and antispermatogenic [34], activities. *Curcuma amada* is distinguished by its pleasant aroma, a feature attributed to its essential oil content. These oils exhibit seasonal variations [36], and have been analyzed using gas chromatography-mass spectrometry (GC-MS) techniques [35, 37]. The compound alpha-pinene within the essential oil significantly contributes to the plant's fragrance. Mango ginger also contains flavonoids, terpenoids, tannins, steroids, alkaloids, and glycosides. The choice of solvent markedly influences the phytochemical properties of mango ginger extracts. An ethanol extract from mango ginger rhizomes identified glycosides, resins, phytosterols, and tannins as the predominant compounds, whereas an aqueous extract revealed only phytosterols and glycosides. The primary components of mango ginger essential oil include myrcene, ocimene, arturmerone, (Z)-β-farnesene, guaia-6, 9-diene, cis-β-ocimene, cis-

hydroocimene, transhydroocimene, α-longipinene, α-guaiene, linalool, β-curcumene, and turmerone [38]. The rhizomes also contain curcumin, bis-demethoxycurcumin, and demethoxycurcumin. Another study identified cis- and transhydroocimene, ocimene, and myrcene as the main aroma-influencing compounds in mango ginger essential oils. Mango ginger consists of 6.39% starch by fresh weight and 45.64% by dry weight. Recent findings indicate that mango ginger rhizomes contain various fatty acids, as well as caffeic, gentisic, ferulic, gallic, cinnamic, protocatechuic, p-coumaric, and syringic acids. A separate study using GC-MS to analyze the dichloromethane extract of mango ginger identified several chemical components, including curcumene, α-curcumene, ß-curcumene, camphor, curzerenone, 1, 8-cineole, curcumin, demethoxycurcumin, bis-demethoxycurcumin, caffeic acid, ferulic acid, gallic acid, cinnamic acid, p-coumaric acid, and gentisic acid.

Materials and Methods

Chemicals and reagents

The study utilised analytical-grade chemicals and reagents procured from reputable companies and institutions, including Sd-fine chemicals, SIGMA, SRL (India), MERCK, RANBAXY, and SUYOG. The materials included silica gel (G), 60F, and 0.25 pre-made aluminium sheets from Merck KGaA, Darmstadt, Germany. Furthermore, silica gel 60 F254, HPTLC aluminium sheets measuring 20×20 cm were obtained from Merck KGaA, Germany.

Sample collection

The rhizomes were procured from Punshari Shop in Jaipur, India and identified by Mr. Vinod Kumar Sharma from the Department of Botany, University of Rajasthan. The rhizomes were washed, dried in shade, and ground into fine powder using an electric blender. The powder was sieved and stored in an airtight container for experimental use.

Extraction of phytochemicals

The sample powder was defatted using petroleum ether, then extracted with 500 mL of methanol in a Soxhlet apparatus. This 8-hour extraction process used chloroform, acetone, ethyl acetate, and methanol. The extract was concentrated by evaporating the solvent using a rotary vacuum evaporator and dried in Petri dishes, then stored in screw-cap tubes at 4°C. The defatted plant material (100 g) underwent percolation with 95% alcohol for 16 hours, repeated thrice until exhausted.

Column Chromatography: A column (2 inches diameter, 95 inches length) was prepared with silica gel bed and petroleum ether solvent, settling for 30 minutes. A mixture of 10 grams ethyl acetate extract and 5 milliliters toluene was added to the silica gel bed, maintaining 1 cm solvent level. Fractions were collected using petroleum ether, chloroform, and ethyl acetate as eluting solvents. The fractions underwent purification through thin-layer chromatography using petroleum ether and ethyl acetate over silica gel.

Thin layer chromatography (TLC)

Thin-layer chromatography was performed using silica gel 60F254 (2 x 5 cm, 0.25 mm thickness) from Merck. KDM extract (25 mg/mL) was filtered through a 0.45-micron filter, and 25 microliters was applied to a TLC plate. The plate was developed for 20 minutes using petroleum ether and ethyl acetate (20:3) as mobile phase, dried at 68°C, and examined under UV at 254 and 360 nm. Colored spots were observed under UV light. Dark brown bands emerged in an iodine chamber, which were scraped and suspended in mobile phase for 3-4 days. After vacuum evaporation, the residue was analyzed using NMR, HR-MS, and IR spectroscopy.

Identification of bioactive compounds by HR-MS, FT-NMR, and FT-IR

The molecular weights were ascertained through High-Resolution Mass Spectrometry (HR-MS). Compound identification was conducted using Fourier Transform Nuclear Magnetic Resonance (FT-NMR) with a JEOL JNM 500 MHz instrument, incorporating both proton (1H NMR) and carbon (^{13}C NMR) analyses. A pure isolate (3 mg) was dissolved in deuterated solvent (CD_3OD) and placed in an NMR tube within the magnetic field tank. The functional groups of the compounds were identified using Fourier-transform infrared spectroscopy (FT-IR) (Bruker-Tension II), with a scan range of 4000 cm^{-1} to 500 cm^{-1} at a resolution of 4 cm^{-1} over a duration of 45 seconds.

Results and Discussion

Utilizing specified extraction and chromatography methodologies, we successfully isolated two novel compounds. Methanol extraction facilitated the separation of compounds from the plant rhizome, which were subsequently purified through thin-layer chromatography (TLC). Among the four solvents employed-petroleum ether, chloroform, and ethyl acetate-the highly polar ethyl acetate proved most effective in extracting medicinally significant secondary metabolites, including alkaloids, flavonoids, and phenols, from Curcuma amada rhizomes. The mobile phase for TLC was optimized using various solvent mixtures, with petroleum ether and ethyl acetate being selected as the most suitable mobile phase. Detection of the spots was achieved at UV wavelengths of 254 and 360 nm. The phytoconstituents in the ethyl acetate extract exhibited visible colored spots on the TLC plate under UV light following treatment with spraying reagents. The identified spots were scraped off and dissolved in methanol for further analysis. Similar ethyl acetate fractions were collected, and the solvent was evaporated to yield a partially purified compound.

Spectral analysis

NMR spectral analysis

Table 1 displays the shielding and deshielding values for ^{1}H and ^{13}C NMR of the compounds. The final structures were verified using ^{13}C NMR spectroscopy, and the determined structure is illustrated in Fig. 1. The ^{13}C NMR spectra for the newly isolated compounds are presented in Fig. 2.

Table 1: ^{1}H NMR and ^{13}C NMR spectral data of compound 1 & 2 (400 MHz, $CDCl_3$, δ- values)

Carbon Number	Compound 1		Compound 2	
	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR
C-1	76.79	5.09 (dd, J= 1.6)	76.79	5.09 (t, J= 7.2)
C-2	138.76	7.007 (d, J= 8.0)	138.76	7.005 (d, J= 7.2)
C-3	153.71	6.67 (dd, J= 8.0, 1.2)	153.71	6.67 (d, J=7.6)
C-4	183.94		183.94	
C-5	161.83		161.83	
C-6	120.47	6.60 (s)	120.11	6.60 (s)
C-7	147.3		147.37	
C-8	161.83		161.83	
C-9	113.60	113.60	113.50	
C-10	131.52	131.52	131.51	
C-1`	168.20		168.20	
C-2`	50.9	2.62 (m)	50.9	2.62 (m)
C-3`	38.4	1.22 (m)	38.46	1.22 (m)
C-4`	39.1	1.59 (m)	41.83	1.55 (m, J= 6.0)
C-5`	29.4	1.86 (m, J= 5.2)	72.40	
C-6`	17.78	1.22(d, J=5.6)	23.39	1.18 (s)
C-1``	15.4	1.32 (d, J= 5.2)	15.4	1.32 (d, J=5.2)
C-2''	22.4	1.26 (m)	22.4	0.87 (d, J=6.0)
C-3''	14.22	1.18 (t, J= 6.8)	26.6	1.19(s)
C-4''	16.4	1.24(d, J=5.6)	22.17	2.2 (s)
C-5''	168.20			

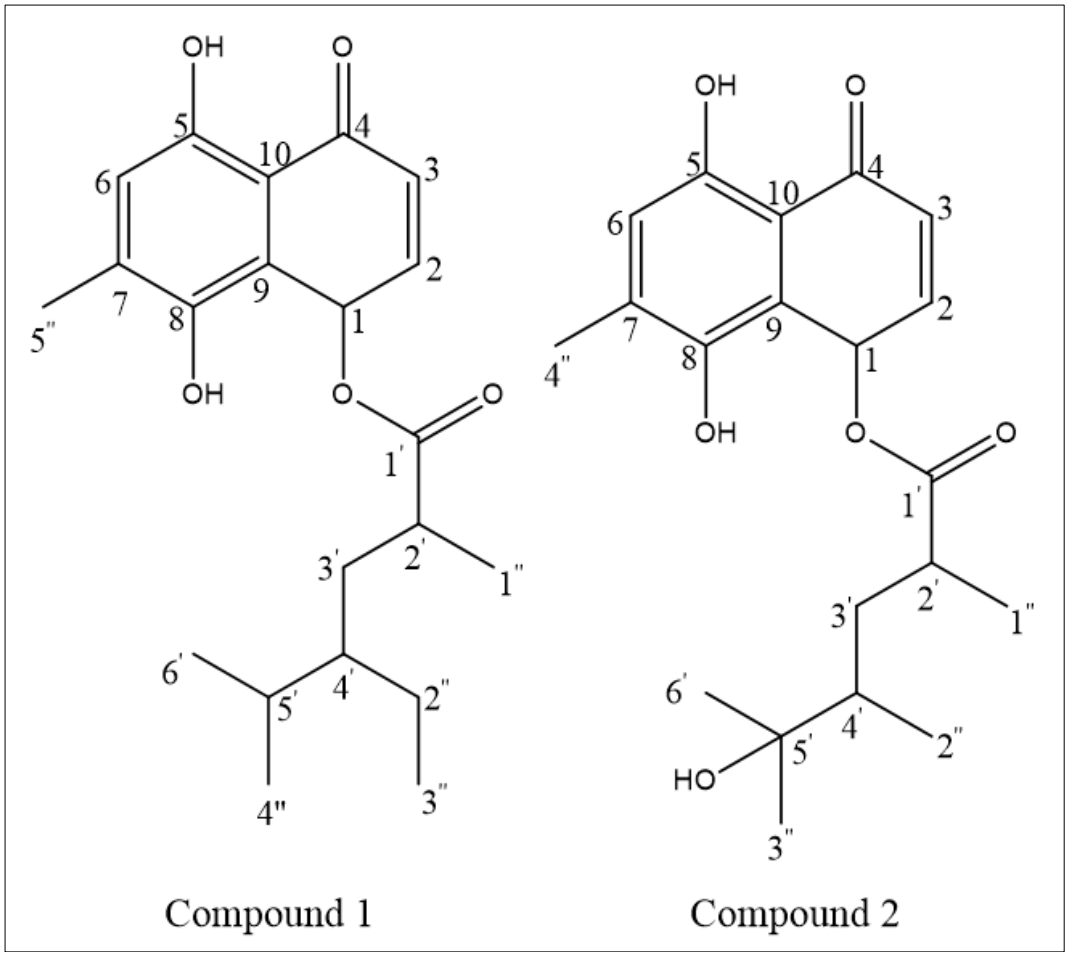
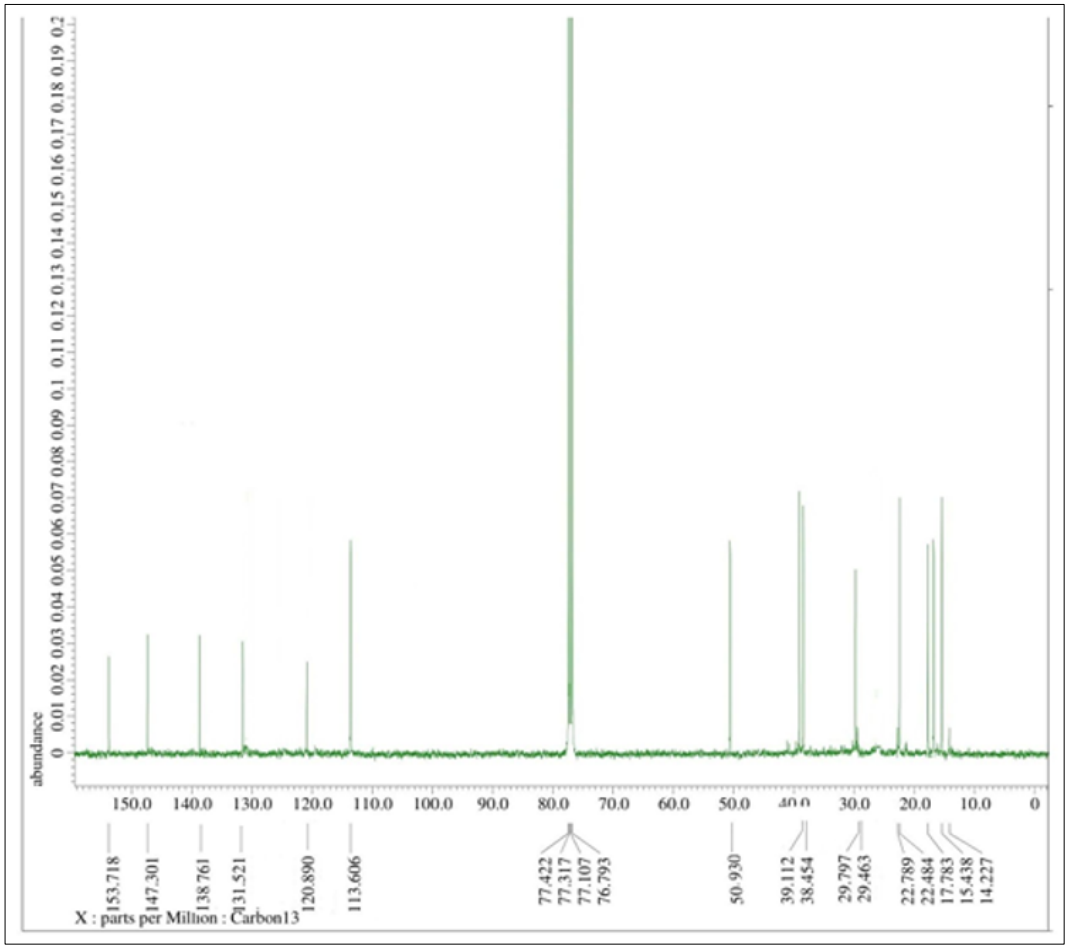
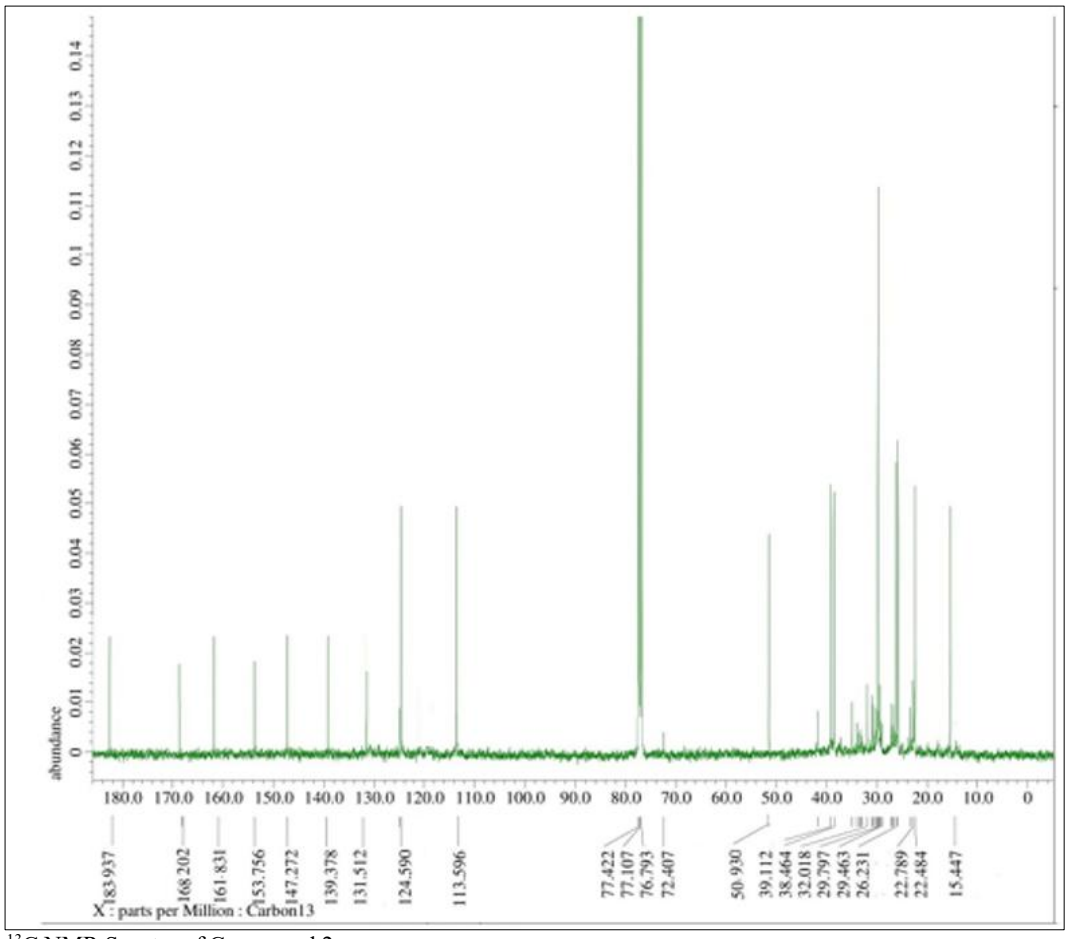


Fig 1: Structure of compounds 1 and 2



^{13}C NMR Spectra of Compound 1



^{13}C NMR Spectra of Compound 2

Fig 2: ^{13}C NMR Spectra of Compounds 1 and 2

Elucidated structure and Name of the novel compounds identified in *Curcuma amada*

The configuration and identification of the newly discovered compound in *Curcuma amada* are shown in Fig. 1. The fractions were purified using column chromatography and thin-layer chromatography (TLC). Upon elution with petroleum ether and ethyl acetate (9:1), compound 1 (5, 8-dihydroxy-7-methyl-4-oxo-1-hydronaphthalen-1-yl 4'-ethyl-2', 5'-dimethylhexanoate) was isolated as a dark brown sticky substance. The mass spectrum showed a molecular ion peak [M+] at 360.16, corresponding to formula $C_{21}H_{28}O_5$. The IR spectra showed absorptions at 1690 cm^{-1} (CO stretching), 1625-1512 cm^{-1} (C=C bands), and 1280 and 1150 cm^{-1} (C-O bending and stretching). The 1H NMR spectrum displayed an olefinic proton doublet at δ 6.67 ppm (J= 8, 1.2 Hz) for H-39. A double doublet at δ 7.007 ppm (J= 8 Hz) was observed for H-2, due to coupling with H-3 and H-1. H-1 showed a double doublet at δ 5.07 ppm (J=1.6) from allylic coupling. A singlet at δ 6.60 ppm was attributed to H-6 in a naphthoquinone-type skeleton. A singlet at δ 2.2 ppm indicated the methyl proton on C-7.

Upon elution with petroleum ether and ethyl acetate (17:3), compound 2 (8-dihydroxy-7-methyl-4-oxo-1-hydronaphthalen-1-yl 5'-hydroxy-2', 4', 5'-trimethylhexanoate) was isolated as a dark brown sticky substance. The ^{1}H NMR spectrum of compound 2 (Table 1) was partially similar to compound 1. HRMS indicated a molecular-ion peak at m/z 362.14, suggesting composition $C_{20}H_{26}O_6$. IR spectra showed absorption at 1692 cm^{-1} (CO stretching), 1620-1509 cm^{-1} (C=C bands), and at 1276 and 1145 cm^{-1} (C-O bending and stretching). The 1H NMR spectrum showed an olefinic proton doublet at δ 6.67 ppm (J= 7.6 Hz) for H-39, a doublet at δ 7.005 ppm (J= 7.2 Hz) for H-2, and a triplet at δ 5.09 ppm (J=7.2) for H-1. A singlet at δ 6.60 ppm was assigned to H-7 in a naphthoquinone-type skeleton. A singlet at δ 2.2 ppm indicated the methyl proton on C-7. A multiplet at δ 2.62 ppm appeared for the C-2' proton. Two singlets at δ 1.18 and δ 1.19 ppm represented six protons of two methyl groups on C-5'. In 13C spectra, δ 72.4 was assigned to C-5' carbon with a hydroxyl group.

Conclusion

In conclusion, this study represents the first successful extraction of two distinct compounds from the rhizomes of Curcuma amada. The molecular structures of these compounds were determined using nuclear magnetic resonance (NMR), infrared (IR) spectroscopy, and mass spectrometry. These compounds, labeled as 1 and 2, were characterized, and their structures elucidated through comprehensive spectroscopic analysis. The ^{13}C NMR spectra of both compounds (Figure 2) provide essential information for structural determination. Further investigation into these compounds may reveal their biological activities and potential applications in fields such as pharmaceuticals and natural product research.

Conflict of Interest declaration: The author declare that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

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