

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/13700499>

# Novel Bioactivities of Curcuma longa Constituents

**Article** in *Journal of Natural Products* · May 1998

DOI: 10.1021/np970459f · Source: PubMed

---

CITATIONS

213

READS

214

**3 authors**, including:



**Muraleedharan Gopalan Nair**

Michigan State University

**247** PUBLICATIONS **13,591** CITATIONS

SEE PROFILE

**Some of the authors of this publication are also working on these related projects:**



Dry bean research [View project](#)



bioactive natural products for human health [View project](#)

## Novel Bioactivities of *Curcuma longa* Constituents

Geoffrey N. Roth, Amitabh Chandra,<sup>†</sup> and Muraleedharan G. Nair\*

Bioactive Natural Products Laboratory, Department of Horticulture and National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48823

Received October 8, 1997

Bioassay-directed fractionation of ethyl acetate extract from *Curcuma longa* Linn. rhizomes yielded three curcuminoids, which displayed topoisomerase I and II enzyme inhibition activity. Curcumin III (**3**) was the most active curcuminoid, inhibiting topoisomerase at 25  $\mu\text{g mL}^{-1}$ . Curcumin I (**1**) and curcumin II (**2**) inhibited the topoisomerases at 50  $\mu\text{g mL}^{-1}$ . Fractionation of the volatile oil from the rhizomes afforded *ar*-turmerone (**4**), which displayed mosquitocidal activity with an LD<sub>100</sub> of 50  $\mu\text{g mL}^{-1}$  on *Aedes aegyptii* larvae. Bioassay-directed fractionation of hexane extract from the turmeric leaves yielded labda-8(17),12-diene-15,16-dial (**5**) with antifungal activity against *Candida albicans* at 1  $\mu\text{g mL}^{-1}$  and inhibited the growth of *Candida krusei* and *Candida parapsilosis* at 25  $\mu\text{g mL}^{-1}$ . In addition, **5** displayed 100% mosquitocidal activity on *A. aegyptii* larvae at 10  $\mu\text{g mL}^{-1}$ .

The tropical plant, *Curcuma longa* L. (Zingiberaceae), native to south and southeast tropical Asia, has a long and distinguished human use in Eastern civilization. In contrast to turmeric's limited use in Western countries as a coloring agent, most turmeric is widely consumed in the countries of origin for a variety of uses. Almost every religious Hindu ceremony makes use of turmeric in one form or another.<sup>1</sup>

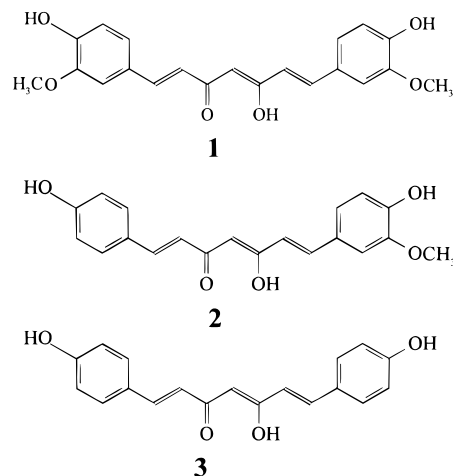
In Indian folk medicine, turmeric has been used to combat a variety of ailments.<sup>2</sup> Many of its reputed medicinal properties have been substantiated. Turmeric has been used as a fever alleviator, and it has potential in cancer prevention.<sup>3,4</sup> Feeding turmeric to mice prevented tumor formation normally caused by benzopyrene, 3-methylcholanthrene, and 3'-methyl-4-(dimethylamino)benzene.<sup>3,4</sup> Turmeric inhibited mutagenicity of cigarette smoke condensates and tobacco extracts.<sup>5</sup> Curcumin also has potential as an antiviral agent. It has been proven a modest inhibitor of HIV-1 and HIV-2 proteases.<sup>6</sup>

Considering the potential of isolating additional bioactive compounds from *C. longa*, our laboratory conducted a bioassay-directed isolation and purification of active components from its rhizomes and leaves. We report novel biological activities for the turmeric metabolites from the rhizomes and the isolation of a diterpene antibiotic, labda-8(17),12-diene-15,16-dial, from the leaves of *C. longa*.

Preliminary bioassays were carried out on all extracts at 250  $\mu\text{g mL}^{-1}$  concentrations to determine the presence of antibacterial, antifungal, mosquitocidal, and nematocidal (*Panagrellus redivivus* Goody, *Caenorhabditis elegans*) activities.<sup>7</sup> Similarly, all crude extracts were tested for topoisomerase inhibition, using mutant *Saccharomyces cerevisiae* cultures.<sup>8,9</sup> These crude extracts also were evaluated for growth inhibition of gypsy moth larvae (*Lymantria dispar*), forest tent caterpillar

larvae (*Malacosoma dystria*), tobacco hornworm larvae (*Manduca sexta*), and corn earworm larvae (*Helicoverpa zea*).<sup>7</sup> Results indicated that only the EtOAc extract from the rhizomes inhibited the growth of *S. cerevisiae* JN394, JN494t<sub>-1</sub>, and JN394t<sub>2-5</sub>. The crude hexane and MeOH extract from the rhizome did not show antimicrobial, insecticidal, or insecticidal activities. However, the volatile oil from the rhizomes demonstrated mosquitocidal activity.

Purification of the EtOAc fraction afforded top-I and top-II active compounds **1,2**, and **3**. Their structures were confirmed by <sup>1</sup>H- and <sup>13</sup>C NMR and EIMS experiments as curcumin I (**1**), curcumin II (**2**), and curcumin III (**3**).<sup>10</sup> The volatile oil of the rhizome yielded a mosquitocidal compound and its structure was confirmed by <sup>1</sup>H-, <sup>13</sup>C NMR and EIMS data as a sesquiterpene ketone, *ar*-turmerone (**4**).

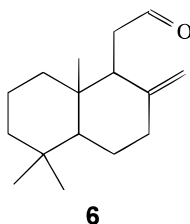
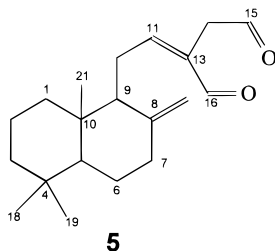
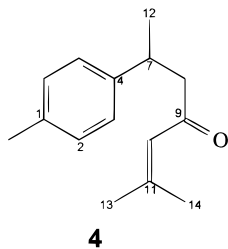


The hexane extract from the leaves exhibited good antifungal and mosquitocidal activities. The crude leaf MeOH and EtOAc extracts did not give antimicrobial, insecticidal, anticancer, or insecticidal activities. Bioassay-directed fractionation of the hexane extract yielded an antifungal compound **5**. The <sup>1</sup>H and <sup>13</sup>C NMR

\* To whom correspondence should be addressed. Tel.: (517) 353-2915. Fax: (517) 432-2242. E-mail: nairm@pilot.msu.edu.

<sup>†</sup> Present Address: Nutraceutical Corp., 1104 Country Hills Dr., Suite 300 Ogden, UT 84403.

spectral data of **5** were identical to that of the known compound labda-8(17)-diene-15,16-dial.



The molar ellipticity of compound **5** was at the extremum of  $-17.23$  mdeg at  $320$  nm. Compound **5** with a  $(-)$  optical rotation was isolated previously from the seed of *Alpinia galanga*<sup>11</sup> and the  $(+)$  isomer from the rhizomes of *Alpinia speciosa*.<sup>12</sup> Also, the absolute configuration of (17),12-labdadiene-15,16-dial from *A. galanga* was determined by the ozonolysis of the diterpene 8(17),12-labdadiene-15,16-dial, yielding compound **6**.<sup>11</sup> The CD spectrum of **6** gave a molar ellipticity of  $-2.79$  at  $289$  nm.<sup>8</sup> The sign value of the ellipticity would be the same for compound **6** as in the case of the natural product from *A. galanga*, since no chiral centers were lost or introduced during the ozonolysis. The negative value of the ellipticity of compound **5** indicated that both compound **5** and 8(17),12-labdadiene-15,16-dial from *A. galanga* are identical in their configuration. Also, due to the double bond and aldehydic group in conjugation, a higher ellipticity value was obtained for **5** than for **6**. This is the first report of the CD for labda-8(17)-12-diene-15,16-dial.

Curcumin I (**1**), curcumin II (**2**), and curcumin III (**3**) were evaluated for the inhibition of top-I and top-II enzymes. Compound **3** gave the most potent topoisomerase I and II enzyme inhibitory activity at  $25 \mu\text{g mL}^{-1}$  concentration. However, **2** and **3** inhibited topoisomerase I and II only at  $50 \mu\text{g mL}^{-1}$  concentration. In mosquitocidal assays, compound **4** displayed an  $\text{LD}_{100}$  at  $50 \mu\text{g mL}^{-1}$  when tested on *A. aegyptii*.

Compound **5** was active against *Candida albicans* at  $1 \mu\text{g mL}^{-1}$ . Also, it inhibited the growth of *Candida krusei* and *Candida parapsilosis* at  $25 \mu\text{g mL}^{-1}$ . It gave 100% mortality at  $10 \mu\text{g mL}^{-1}$  when tested on 4th instar *A. aegyptii* larvae. The most effective mosquitocidal component was compound **5** when compared to the

activity of *ar*-turmerone, compound **4**. Interestingly, *ar*-turmerone caused mortality in 18 h at  $50 \mu\text{g mL}^{-1}$ , while compound **5** gave 100% mortality at 48 h.

The mechanism for the biological activity of these compounds has not been determined. However, some observations regarding inhibition displayed by the curcuminoids, **1–3**, can be made on the basis of their structural differences. Compound **3** displayed greater top-I and top-II inhibitory activity than either **2** or **3**. The lack of  $\text{OCH}_3$  groups probably is responsible for the increased activity of **3**.

Curcuminoids are widely used by the food industry as a safe coloring agent. This is the first report of the topoisomerase activity of curcuminoids (**1–3**) and the isolation and characterization of labda-8(17),12-diene-15,16-dial (**5**) from *C. longa*. Similarly, the biological activities reported in this paper are novel.

## Experimental Section

**Plant Materials.** Turmeric plants were grown in large plastic pots, using a mixture of 50% loamy field and 50% bacto soils. Several rhizomes were planted 3 in. below the surface of the soil in each pot. The plants were grown in the BNPL greenhouse at Michigan State University. The *C. longa* plants were watered every 2–3 days and fertilized with 20–20–20 Peters brand fertilizer. A voucher plant specimen was filed with the Beal-Darlington Herbarium, Department of Botany and Plant Pathology, Michigan State University. The turmeric plants were harvested for the rhizomes every 9 months. Rhizomes were collected, washed with water, lyophilized, milled, and kept in the  $-20^\circ\text{C}$  freezer in Ziploc bags. Some of the fresh rhizomes were replanted for additional production. The turmeric plants were harvested for rhizomes in 9-month cycles. Leaves were collected every 2 weeks for a 3-month period, lyophilized, and milled and kept in a  $-20^\circ\text{C}$  freezer until extraction.

**General Experimental Procedures.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded at the Max T. Rogers NMR facility at Michigan State University on Varian VXR 300 and 500 MHz spectrometers at ambient temperature. Mass spectra were acquired at the Michigan State University Mass Spectrometry Facility on a JEOL HX-110 double-focusing mass spectrometer. Reversed-phase preparative TLC plates were purchased from Whatman Inc. Media ingredient for bioassays were purchased from Difco Lab. The anticancer standards camptothecin and etoposide were purchased from Aldrich Chemical Co.

**Cell Culture Media.** YMG medium (yeast extract 4 g, maltose 10 g, glucose 4 g, and agar  $12 \text{ g L}^{-1}$ ), PDA (potato dextrose agar; potato infusion 200 g, dextrose 20 g, and agar  $15 \text{ g L}^{-1}$ ), Emmons medium (neopeptone 10 g, glucose 20 g, and agar  $15 \text{ g L}^{-1}$ ) NG medium (NaCl 3 g, bacto peptone  $2.5 \text{ g L}^{-1}$ , cholesterol  $1 \text{ mL L}^{-1}$  of  $5 \text{ mg mL}^{-1}$  stock solution,  $\text{CaCl}_2$   $1 \text{ mL L}^{-1}$  of 1 M stock solution,  $\text{MgSO}_4$ ,  $1 \text{ mL L}^{-1}$  of 1 M stock solution, and potassium phosphate buffer  $25 \text{ mL L}^{-1}$  of stock solution of  $\text{KH}_2\text{PO}_4$   $11.97 \text{ g}^{-1}100 \text{ mL}^{-1}$  and  $\text{K}_2\text{HPO}_4$   $2 \text{ g}^{-1}100 \text{ mL}^{-1}$ ), and YPDA medium (yeast extract 20 g, peptone 10 g, dextrose  $20 \text{ g L}^{-1}$ , and adenine sulfate  $2 \text{ mL L}^{-1}$  of  $0.5\%$  stock solution) were prepared as published.<sup>7</sup> Sterile saline was prepared by dissolving NaCl ( $8.5 \text{ g L}^{-1}$ ) in deionized water.

**Isolation and Identification of Topoisomerase Inhibitors 1–3.** Lyophilized and pulverized turmeric rhizomes (100 g) were extracted successively with hexane, ethyl acetate, and methanol in an extraction column. Each solvent was used in 500 mL aliquots ( $\times 3$ ) over a 24 h period. Each extract was evaporated to dryness, yielding 1.11, 4.75, and 5.5 g of hexane, EtOAc, and MeOH extracts, respectively. Only the EtOAc extract exhibited activity on the topoisomerase enzymes, and hence, it was further purified. The EtOAc extract (1.6 g) was initially purified by VLC using silica gel (71.6 g). The mobile phases used were  $\text{CHCl}_3$ , 1:1  $\text{CHCl}_3$ –MeOH, and 100% MeOH. Fractions collected were 1–18 (50 mL each) for  $\text{CHCl}_3$ , 19–23 (50 mL each) for 1:1  $\text{CHCl}_3$ –MeOH, and 24–29 for MeOH (100 mL each). On the basis of TLC analysis, fractions 1–3, 4–6, 7–9, 10–11, 12–18, 19–20, 21–22, and 23–29 were combined to form fractions I–VIII, respectively, and active fractions VII and VIII were combined to yield fraction A (914 mg). Purification of fraction A (267 mg) by preparative TLC using a 30:1  $\text{CHCl}_3$ /MeOH mobile phase yielded bands A–E. The top-I and top-II active bands B, C, and D were collected separately, were further purified by TLC using a 9:1  $\text{CHCl}_3$ /acetic acid mobile phase, and afforded pure compounds **1** (79 mg), **2** (43 mg), and **3** (29 mg), respectively. The % dry weights of **1–3** were calculated as 0.8, 0.4, and 0.3, respectively.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compounds **1–3** were identical to NMR spectra of curcumin I, curcumin II, and curcumin III, respectively.<sup>13</sup>

**Isolation and Characterization of Mosquitocidal Compound 4.** A colorless oil was extracted with ether from the melted ice on the condenser coil of the lyophilizer when fresh rhizomes were freeze-dried. Purification of this colorless oil (465 mg) by TLC using a 50:1 hexane/acetone mobile phase yielded fractions A–E. The fraction C (40 mg) exhibited mosquitocidal activity and was identified as *ar*-turmerone from its  $^1\text{H}$  and  $^{13}\text{C}$ NMR and EIMS spectral data.

**Isolation and Characterization of the Antifungal Compound 5.** Lyophilized and milled turmeric leaves (100 g) were extracted successively with hexane, ethyl acetate, and methanol in an extraction column. Each solvent was used in  $3 \times 500$  mL aliquots over a 24 h period. Each extract was evaporated to dryness, yielding 6, 2, and 15 g of hexane, EtOAc, and MeOH extracts, respectively. Only the hexane extract exhibited antimicrobial activity when tested against a series of fungi, bacteria, and yeast as mentioned later. The crude hexane extract (2.5 g) was dissolved in hexane (300 mL) and partitioned with MeOH ( $3 \times 100$  mL). The MeOH extracts were combined. The hexane and MeOH fractions were evaporated to dryness separately, yielding a light yellow oil and a dark green solid, respectively. The antifungal activity was in the MeOH-partitioned fraction (2.1 g). Chlorophyll was precipitated from this fraction using  $\text{MeOH:H}_2\text{O}$  (7:3) (14 mL  $\times 3$ ), and the supernatant was evaporated to dryness in vacuo to yield a brown oil (1.38 g). A portion of this oily residue (560 mg) was purified by VLC using  $\text{MeOH:H}_2\text{O}$  (85:15) as the mobile phase. The fractions were collected in aliquots of 50 (I), 50 (II), 25 (III), 30 (IV), 35 (V), 40 (VI), and 40 (VII) mL, respectively. Bioassays revealed that fractions III–VII were antifungal and were combined

to yield an oily residue (128 mg). Further purification of this oily residue by reversed-phase preparatory TLC, using  $\text{MeOH:H}_2\text{O}$  (70:30) mobile phase, afforded an oily compound **5** (22 mg). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound **5** were identical to the spectra of the diterpene aldehyde, labda-8(17),12-diene-15,16 dial.

**Circular Dichroism (CD) of Compound 5.** The CD analysis of compound **5** was carried out using a JASCO J-710 71CD-ORD spectropolarimeter. Nitrogen (99.99%) was generated by a nitrogen generator model NG-150 at a rate of 15 L  $\text{min}^{-1}$ . Compound **5** was dissolved in EtOH (1.4 mg  $\text{mL}^{-1}$ ), and the CD was determined under the following conditions: scan mode (wavelength), bandwidth (0.5 nm), sensitivity (50 m deg), response (1 s), wavelength range (200–400 nm), step resolution (1 nm), scan speed (200 nm  $\text{min}^{-1}$ ), and accumulation (1).

**Antimicrobial Assays of Compounds 1–5.** The assays were conducted according to the reported procedure.<sup>7</sup> Cultures of *Fusarium oxysporum* (MSU-SM-1322), *Fusarium moniliforme* (MSU-SM-1323), *Botrytis* spp., *Gleosporium* spp., and *Rhizoctonia* spp. were raised on PDA medium in Petri dishes. Cultures of *C. albicans* and *Aspergillus flavus* (MSU strains) were cultured on YMG medium in Petri dishes. Cultures of *Staphylococcus epidermidis* (ATCC 25923), *Streptococcus pyogenes* MHM-1645, *Streptococcus aureus* (MS stain), and *Escherichia coli* (ATCC 25922) were grown on Emmons medium in Petri dishes.

**Topoisomerase Inhibition Assay.** Compounds **1–5** were evaluated for top-I and top-II inhibition according to the reported procedure.<sup>8</sup> Mutant *Saccharomyces cerevisiae* cell cultures JN394, JN394t<sub>-1</sub>, and JN394t<sub>2-5</sub> were provided by Dr. John Nitiss at St. Jude Children's Research Hospital. JN394 is hypersensitive to topoisomerase I and II poisons because of deletions that deleted the RAD52 repair pathway. JN394t<sub>-1</sub> is isogenic to JN394 except that the top-I gene is deleted. The deletion of this gene causes a lack of response to topoisomerase I poisons. JN394t<sub>2-5</sub> that contains the top-II gene is resistant to topoisomerase II poisons, but it responds to topoisomerase I poisons. Topoisomerase I and II are enzymes that alter the DNA by catalyzing a three-step process. This involves cleavage of one strand of DNA by topoisomerase I or two strands of DNA by topoisomerase II, movement of a DNA segment through this break, and then resealing of the DNA strand(s).<sup>9</sup> The mutant yeasts were raised on YPDA medium in Petri dishes.

The cell suspension for bacteria and mutant yeast and spore suspensions for fungi were prepared by suspending the bacteria or yeast or spores from a fully grown culture in a Petri dish in 10 mL of sterile saline solution and transferring the suspension to a sterile culture tube. The cell or spore suspension concentration was adjusted to  $10^6$  colony-forming units per milliliter (CFU  $\text{mL}^{-1}$ ) to yield a stock solution. Serial dilutions of the stock culture were made in sterile saline by inoculating the proper media with the approximate dilutions to determine the required CFU  $\text{mL}^{-1}$ . Bioassays were conducted by spreading 100  $\mu\text{L}$  of the desired cell or spore suspension contain  $10^6$  CFU  $\text{mL}^{-1}$  on Petri dishes of the corresponding medium. The test compound was spotted carefully on the bioassay plates at varying concentra-

tions, along with 20  $\mu\text{L}$  of DMSO alone as control. The plates were allowed to dry in a laminar flow hood and then incubated at 27 °C for 72 h. The zone of inhibition was measured in mm. Minimum inhibitory concentration (MIC) was determined for compounds **1–3** according to the published procedure.<sup>7</sup>

**Mosquitocidal Assays.** *A. aegyptii* mosquito larvae were used to test for mosquitocidal activity of compounds **1–5** according to the published procedure.<sup>7</sup> *A. aegyptii* eggs were provided through the courtesy of Dr. Alex Raikhel of the Department of Entomology at Michigan State University. Eggs were placed in 500 mL of degassed, deionized water prepared by sonication. About 5 mg of bovine liver powder was added to the water as a food source. After 4 days, 4th instar mosquito larvae were transferred to 980  $\mu\text{L}$  of the water in a test tube. Twenty microliters of the test material at the desired concentration in DMSO was added to each tube, which was shaken lightly to ensure a homogeneous test solution. Each tube was covered and left at room temperature. Twenty microliters of DMSO was used as the control. The larval mortality was recorded at 2, 4, 6, 24, and 48 h intervals. The assay was conducted in triplicate.

## References and Notes

- (1) Kurup, P. N. V. *Handbook of Medicinal Plants*; Central Council for Research in Ayurveda and Siddha: Lucknow, India, 1979; p 78.
- (2) Lindley, J. *Flora Medica: A botanical account of all the more important plants used in medicine*; Longman, Orme, Brown, Green and Longmans: London, 1838; p 562.
- (3) Polasa, K.; Sesikaran, B.; Krishna, T.; Krishnamurthy, K. *Fd Chem. Toxic.* **1991**, *29*, 699–706.
- (4) Reddy, A.; Lokesh, B. *Fd. Chem. Toxic.* **1994**, *32*, 279–283.
- (5) Nagabhushan, M.; Arnonkar, A.; Bhide, S. *Food Chem. Toxicol.* **1987**, *25*, 545–547.
- (6) Sui Z.; Salto, R.; Li, J.; Craik, C.; de Montellano, P. *Biorg. Med. Chem.* **1993**, *6*, 415–422.
- (7) Nair M. G.; Putnam, A. R.; Mishra, S. K.; Mulks, M. H.; Taft, W. H.; Keller, B. E.; Miller, J. R.; Zhu, P.-P.; Meinhart, D.; Lynn, D. G. *J. Nat. Prod.* **1989**, *52*, 797–809.
- (8) Chang, Y.-C.; Nair, M. G. *J. Nat. Prod.* **1995**, *58*, 1901–1905.
- (9) Nitiss, J.; Wang, J. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7501–7505.
- (10) Govindarajan, V. *Turmeric-Chemistry, technology and Quality*; CRC Press: Boca Raton, FL, 1980; pp 3, 12.
- (11) Morita, H.; Itokawa, H. *Planta Med.* **1988**, *54*, 117–120.
- (12) Itokawa, H.; Morita, M.; Mihashi, S. *Chem. Pharm. Bull.* **1980**, *34*, 3452.
- (13) Roughly, P. J.; Whiting, D. A. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2379.

NP970459F