Isolation, Purification, and Characterization of Insect Repellents from *Curcuma longa* L.

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Two compounds were isolated from *Curcuma longa* L. and identified from their spectral characteristics as 2-methyl-6-(4-methylphenyl)-2-hepten-4-one (*ar*-turmerone) and 2-methyl-6-(4-methyl-1,4-cyclohexadien-1-yl)-2-hepten-4-one (turmerone). *ar*-Turmerone and turmerone gave an average 62.9% (class IV) and 43.1% (class III) repellency, respectively, to *Tribolium castaneum* (Hbst.) after 8 weeks of study. Turmerone was unstable thermally and also at ambient temperature in the presence of air, yielding its dimer or the more stable *ar*-turmerone.

Tumeric, *Curcuma longa* L., is a tropical herb of the Zingiberaceae family indigenous to southern Asia. The aromatic yellow powder from its mature rhizomes was used in Asian countries for many centuries as a yellow vegetable dye for silks and cottons. It is still used in foods as a condiment, particularly as an essential ingredient of curry powder, in medicine as a stomachic, carminative, anthelmintic, laxative, and cure for liver ailment, and also as an ant repellent in India (Sreenivasamurthy and Krishnamurthy, 1959; Watt and Breyer-Brandwijk, 1962).

The strong coloring pigment in turmeric contains curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, and two other curcuminoids (Srinivasan, 1953; Lubis, 1968; Shankaracharya and Natarajan, 1974; Krishnamurthy et al., 1976). The red-yellow essential oil of turmeric, either from steam distillation or from an oil-selecting solvent extraction, contains *d*-α-phellandrene, *d*-sabinene, zingiberene, borneol, 1,8-cineole, turmerone, *ar*-turmerone, sesquiterpene alcohols, α- and γ-atlantone, and bisabolene (Gildemeister and Hoffmann, 1956; Mima, 1959; Shankaracharya and Natarajan, 1974; Salzer, 1975 Govindarajan, 1980).

Shankar et al. (1980) conducted feeding toxicity studies of turmeric and its alcohol extract in rats, guinea pigs, and monkeys. The results indicated that turmeric is nontoxic even at the very high level tested. There was absolutely no mortality or any morphological and histological abnormalities in the experimental animals at 2.5 g of turmeric/kg of body weight which corresponds to a maximum consumption of 5 g/day for an adult human with an average body weight of 70 kg. It is the practice in some southern Asian countries; i.e., India and Pakistan, to store rice or wheat by mixing it with 2% of turmeric powder (Chatterjee, 1980). In this paper, we report the isolation, purification, and identification of the repellent components of turmeric to *Tribolium castaneum* (Hbst.).

MATERIALS AND METHODS

Extraction of Turmeric. Powdered turmeric (imported from India) was purchased from Lex Enterprise, Atlanta, GA. One kilogram of the powder was extracted in a Soxhlet extractor with petroleum ether (bp 30–60 °C) for 24 h. The petroleum ether solution was concentrated under reduced pressure in a rotary evaporator to obtain the crude turmeric extract.

Liquid Column Chromatographic (LCC) Fractionation of Crude Turmeric Extract. *Step 1.* Each 1.4–1.6-g portion of the crude extract was placed on a column (40 × 2.0 cm i.d.) of silica gel (70–230 mesh; EM Reagent; extra pure) and eluted with chloroform. A total of 120 2-mL fractions was collected. After the solvent was removed, every third fraction was spot tested by TLC (see below for procedure). The fractions possessing the same R_f values were combined, and each combined fraction was bioassayed for repellency to *T. castaneum*. The insect active fraction was further fractionated.

Step 2. Each 150–160-mg sample of the active fraction from step 1 was further separated by column chromatography in a 40 × 1.2 cm i.d. column of silica gel and eluted with benzene. A total of 90 2-mL fractions was collected. TLC analysis was made on every third fraction. The fractions with only R_f 0.25–0.28, the mixture of R_f 0.25–0.28 and 0.30–0.33, and only R_f 0.30–0.33 were combined separately. The two pure combined fractions were bioassayed.

Thin-Layer Chromatographic Analysis of LCC Fractions. Brinkman EM reagent, precoated silica gel G F_{254}, 20 × 20 cm analytical chromatoplates were used. A small sample of each selected fraction was applied on spots 2.5 cm above the lower edge and 1.5 cm from each other. Each plate was developed in benzene and then examined under UV at 254 nm.

High-Performance Liquid Chromatographic Purification. A Waters Associates Model ALC/GPC 244 high-pressure liquid chromatograph with a Model 6000A pump, a U6K injector, an R401 differential refractometer, and a Model 440 UV detector with a 300 × 7.8 mm i.d. μBondapak C_{18} column (octadecyltrichlorsilane covalently bonded to 10-μm μPorasil packing) was used. Methanol–water (8:2 by volume, degassed) was used as the eluting system. The column effluent was monitored at 254 nm (2.0 AUFS), and the response was recorded on a Houston Omniscribe Model B-5217-1 recorder.

Each of the two LCC separated components was dissolved individually in methanol to obtain the concentration of approximately 20 μg/μL and filtered through a Waters Associates sample clarification kit with 0.5-μm Millipore organic filter system. For each run, a 5-μL aliquot of the stock solution was injected onto the column with the flow rate of the eluting solvent at 1.5 mL/min. The desired peak of the compound as indicated from the recorder was recycled through the column once, and the effluent of the recycled peak was collected. The process was repeated

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Table I. Repellency of Turmeric Crude Extract, Compounds 1 and 2, and Curcumin to *T. castaneum* Hbst. Adults

material	concn, $\mu g/cm^2$	% mean repellency at the indicated week after treatment					repellency, class
		1st	2nd	4th	8th	av	
extract	680	92.6	78.5	67.5	47.5	71.5	IV
compound 1	200	75.5	66.0	57.5	52.5	62.9	IV
compound 2	200	43.0	49.0	42.0	38.5	43.1	III
curcumin	200	4.0	-2.0	-1.2	6.5	1.8	I

until sufficient material was collected. The combined effluent from same material was concentrated under reduced pressure to remove methanol and then lyophilized below 0 °C.

Instrumental Analyses. A Du Pont Model 21-490B mass spectrometer was used with a direct insertion probe at a temperature of 150–250 °C (below 70 °C for the thermally unstable compound). Other conditions used were as follows: ion source temperature, 150 °C; ionizing voltage, 70 eV; ion source pressure, 4×10^{-6} torr; scan rate, 100 s/decade from 15 to 500 amu. For deuterium–hydrogen exchange of the sample, the material was mixed with deuterium oxide and dioxane (1:2 by volume) and a catalytic amount of potassium hydroxide. The solution was stirred for 30 min, and then the deuterium oxide and dioxane were removed under vacuum at 50 torr. The remaining oil was dissolved in a minimum amount of methylene chloride and about 0.2 μL was taken up into a glass capillary. After the solvent was evaporated, the mass spectrum was obtained.

NMR spectra were obtained from a JEOL Model FX 60Q NMR spectrometer at ambient temperature. The samples were prepared in $CDCl_3$ with tetramethylsilane as the zero reference. Infrared spectra were determined as thin films with a Beckman 4230 spectrophotometer. The UV spectra were obtained with a Varian Cary 210 spectrophotometer. The samples were prepared in absolute ethanol solution by using cells with 1-cm paths, with wavelengths in nanometers.

Chemicals and Reagents. Curcumin was purchased from Eastman Kodak Co. High-performance LC methanol (Fisher Scientific Co.) was filtered through a Waters Associates solvent clarification kit with a 0.5-μm Millipore organic filtration system. All other solvents were the reagent grade.

Repellency Evaluation Method. The crude extract and LCC components were evaluated for their repellency against *T. castaneum*. The insects were 7–14-day-old adults from a laboratory colony reared at 27 ± 1 °C and 60 ± 5% relative humidity. The procedure of Standard Method Number 3 (Repellency and Attractancy) was described by McDonald et al. (1970) and Gillenwater and McDonald (1975). The crude extract was tested at the concentration of 680 $\mu g/cm^2$, while the LCC compounds 1 and 2 and curcumin were tested at 200 $\mu g/cm^2$. The overall average percent repellency values for 8 weeks were assigned repellency classes by using the following scale: classes 0, I, II, III, IV, and V designated percent repellency values of <0.1, 0.1–20, 20.1–40, 40.1–60, 60.1–80, and 80.1–100, respectively.

RESULTS AND DISCUSSION

Petroleum ether extraction of the powdered turmeric yielded 7.0–7.3% crude extract of orange red oil. When this extract was fractionated by the LCC method step 1 (with silica gel and eluted with chloroform), the yield of the active portion was 52–54%. When this portion was again fractionated by the LCC method step 2 (with silica gel and eluted with benzene), three fractions were obtained. The yields of fraction 1 of compound 1 (TLC R_f 0.25–0.28), fraction 2 of a mixture of compounds 1 and 2 (TLC R_f 0.25–0.33), and fraction 3 of compound 2 (TLC R_f 0.30–0.33) were approximately 18.5, 30, and 31%, respectively. Only compound 1 and compound 2 were used for insect bioassay.

The repellency to *T. castaneum* of the crude extract, compounds 1 and 2, and curcumin are shown in Table I. The crude extract showed strong repellency at a concentration of 680 $\mu g/cm^2$. Curcumin, its coloring material, was not the component responsible for its activity. Compounds 1 and 2 showed good repellency to the insects at the concentration of 200 $\mu g/cm^2$, with the average repellency of classes IV and III, respectively, after 8 weeks of study. Both compounds 1 and 2 were slightly contaminated with some minor impurities and were purified by the HPLC–recycle technique. The pure materials were used for different instrumental spectral analyses.

Compound 1 was obtained as a colorless oil. Its UV absorption showed 221 nm (ϵ 12 010), 238 nm (ϵ 13 400), and 273 nm (ϵ 580). Strong and moderate infrared absorptions were observed in cm^{-1} at 3035, 1511, 1452, and 1112 (aromatic), 2975 (CH), 1695, and 1614 (C=O and C=C conjugated ketone system), 1385 and 1315 (*gem*-dimethyl), and 818 (para-substituted aromatic). The mass spectrum showed the following peaks (relative peak intensity in parentheses): 216 (M^+, 19), 201 (10), 132 (14), 119 (base peak 100), 105 (12), 91 (11), 83 (84), 77 (6), and 55 (24). After deuterium–hydrogen exchange, the mass spectrum showed the following peaks with the relative intensity at 220 (11), 219 (15), 218 (13), 217 (10), 216 (8), 205 (4), 204 (6), 203 (7), 202 (7), 201 (6), 134 (31), 133 (15), 132 (11), 119 (base peak 100), 91 (21), 85 (42), 84 (67), 83 (88), 57 (13), 56 (21), and 55 (25). The ^1H NMR spectrum showed δ ($CDCl_3$) 1.18 and 1.29 (2 s, 3), 1.84 (d, 3, J = 2 Hz), 2.08 (d, 3, J = 2 Hz), 2.29 (s, 3), 2.58 (d, 2, J = 2 Hz), 3.10 (m, 1), 5.99 (m, 1), and 7.08 (s, 4).

Compound I was identified as 2-methyl-6-(4-methylphenyl)-2-hepten-4-one (*ar*-turmerone) which was previously determined by Honwad and Rao (1964) on the basis of the optical rotatory dispersion curve of the hydrocarbon 1-methyl-4-(1-methylpropyl)benzene derived from it. Honwad and Rao (1964) and Alexander and Rao (1973) reported the UV maximum at 238 nm for *ar*-turmerone. Khalique and Das (1968) found the maxima of 222 and 234 nm with a weak shoulder of 273 in the distilled fraction of turmeric oil which was analyzed to be $C_{15}H_{20}O$ of *ar*-turmerone. We also obtained the two major absorption peaks at 221 and 238 nm with a weak shoulder at 273 nm. The IR spectrum was in agreement with that of Mima (1959), and IR and NMR spectra also were consistent with those reported by Crawford et al. (1972), Grieco and Finkelhor (1973), and Vig et al. (1977) for *ar*-turmerone.

The mass ions obtained for compound 1 were identifcal with those reported for *ar*-turmerone by Crawford et al. (1972). After the deuterium–hydrogen exchange process, a maximum of four deuterium atoms were exchanged to give parent ions at 217, 218, 219, and 220. Peaks at 133 and 134 indicated one and two deuterium atoms were exchanged at the carbon atoms α to the carbonyl group, and peaks at 56 and 57 indicated one and two deuterium

atoms were introduced into the isobutylene group. The number of deuterium atoms exchanged by this compound is consistent with the structure of *ar*-turmerone.

Compound **2** was obtained as a pale yellow oil with a faint sweet odor. Its UV absorption showed 234–235 nm (ϵ 17 400). Strong infrared absorption was observed in cm^{-1} at 2940 (CH), 1688 and 1620 (C=O and C=C conjugated ketone system), 1445 and 1380 (*gem*-dimethyl), and 1035 and 880 (substituted unsaturation). The mass spectrum obtained at probe temperature below 70 °C showed the following prominent peaks (relative peak intensity in parentheses): 218 (M^+, 6), 121 (base peak 100), 105 (16), 93 (7), 91 (15), 83 (55), 79 (6), 77 (8), and 55 (35). Masses (m/e) below the 5% level were not shown. The ^1H NMR spectrum showed δ ($CDCl_3$) 0.93 (m, 2), 1.25 (m, 1), 1.57 (m, 8), 1.87 (d, 3, J = 2 Hz), 2.13 (d, 3, J = 2 Hz), 2.30 (m, 3), 4.73 (b, 1), and 6.06 (b, 1).

Compound **2** was identified as 2-methyl-6-(4-methyl-1,4-cyclohexadien-1-yl)-2-hepten-4-one (turmerone). Its UV absorption maximum at 234–235 nm and IR spectrum were in agreement with those reported by Mima (1959), who obtained pure turmerone by treating its stable thiourea adduct with water. The mass spectrum showed the expected fragments with an additional ion at mass 91. The methylcyclohexadiene ion (93) initially formed could lose two hydrogens to form a tropylium ion as postulated by Biemann (1962) and Rhyge and Von Sydow (1963). Since compound **2** and compound **1** possess the same substituted side chain, we could expect them to give some of the same low mass ions. We found both compounds produced the expected prominent ions at 83 and 55. When the probe temperature was increased above 150 °C during analysis, additional mass ions of 216, 132, and 119 were obtained. The aromatization of the methylcyclohexadiene group in turmerone at elevated temperature occurred as expected. According to the report of Rhyge and Von Sydow (1963), they observed the conversion of various thermally unstable monocyclic terpenes into *p*-cymene during GLC–MS analysis when the gas transport system between the GC and the MS was maintained at about 200 °C.

Turmerone was reported to be unstable upon exposure to air and slowly aromatized to *ar*-turmerone (Alexander and Rao, 1973). Khalique and Das (1968) also suggested that the dehydroturmerone (*ar*-turmerone) was an artifact and not a natural component of turmeric oil. We allowed the turmerone to stand at ambient temperature in the presence of air, and the sample was analyzed by MS with the direct insertion probe unheated and the source temperature at 130 °C. The percent ratio of turmerone: *ar*-turmerone before exposure and after 67 and 91 h exposure was 100:0, 37:63, and 0:100, respectively.

We have identified *ar*-turmerone (compound **1**) and turmerone (compound **2**) in turmeric powder which gave strong repellency to *T. castaneum*. Turmeric powder or the crude extract also showed strong repellency to *Tribolium confusum* Jacq. duVal, *Rhyzopertha dominica* (F.), *Sitophilus granarius* L. adults, and *Attagenus megatoma* (F.) larvae (Jilani and Su, 1980). Therefore, turmeric powder or turmeric oily extract should be effective and safe treatments to consider for development as protectants for wheat, rice, and other cereal grains against damage by the above-named insects.

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