Sesquiterpene and Phenylpropanoids from *Curcuma longa*

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Abstract

A sesquiterpene and two phenylpropanoids were isolated from the carbon tetrachloride soluble fraction of a methanol extract of the rhizomes of *Curcuma longa* (Zingiberaceae). The structures of the isolated compounds were elucidated as turmerone (**1**), *trans*-p-coumaric acid (**2**) and *trans*-ferulic acid (**3**) by extensive spectroscopic studies, including high field NMR and GCMS analyses.

Keywords: *Curcuma longa, Zingiberaceae, turmerone*, *trans-p-coumaric acid, trans-ferulic acid.*

Introduction

Curcuma longa (Family- Zingiberaceae, Bengali name- Halud) is a perennial herb that measures up to 1m high with a short stem and distributed throughout tropical and subtropical regions of the world. It is widely cultivated in Asiatic countries such as Bangladesh, India and China (Araujo and Leon, 2001). The multicomponent essential oils of turmeric have antiviral (Kim *et al*., 2009), anti HIV (De Clercq, 2000), antibacterial (De *et al*., 2009), antioxidant (Singh *et al*., 2010) and antimutagenicity (Guddadarangavvanahally *et al*., 2002) properties. Curcumin, a hydrophobic polyphenol derived from the rhizomes of *C. longa* possesses antioxidative, anticarcinogenic (Nishinaka *et al*., 2007; Bar-Sela *et al*., 2010), anti-proliferative, anti-inflammatory (Ravindran *et al*., 2010) and hypolipidemic activities (Babu and Srinivasan, 1997).

Previous phytochemical studies with *Curcuma* species led to the isolation of several sesquiterpenes such as wenyujinlactone A, neolitamone A, zedoarondiol, isozedoarondiol, aerugidiol, curcumol, curdione, (1R,10R)-epoxy-(-)-1,10-dihydrocurdine (Wang *et al*., 2007) and parviflorene F (Ohtsuki *et al*., 2008) and some curcuminoids e.g., curcumin, demethoxycurcumin and bisdemethoxycurcumin (Pozharitskaya *et al*., 2008).

We, herein, report the isolation of turmerone (**1**), *trans*-p-coumaric acid (**2**) and *trans*-ferulic acid (**3**) from the carbon tetrachloride soluble fraction of a methanol extract of *C. longa*.

Materials and Methods

General experimental procedure

The ^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument and the spectra were referenced to the residual nondeuterated solvent signal. PTLC was carried out using Merck Si gel 60 F_{254} on glass plates (20cm X 20cm) at a thickness of 0.5mm. TLC was conducted on normal-phase Merck Si gel 60 F_{254} on glass plates and spots on TLC and PTLC plates were visualised under UV light at 254nm as well as by spraying with vanillin sulfuric acid followed by heating for 5 minutes at 110ºC.

Collection of Plant Materials

Rhizomes of *C. longa* were collected from Dhaka in the month of February 2008. A voucher specimen for this collection has been deposited in the herbarium of the Department of Botany, University of Dhaka. The samples were cut into small pieces and sun dried for 7 days followed by oven drying for 24 hours at 40°C to facilitate grinding.

Extraction and isolation

The powdered material (533g) was soaked in 1.5 liter of methanol in a large conical flask for 7 days with occasional shaking and stirring. The whole mixture was then filtered off through a cotton plug followed by Whatman filter paper no.1 and the filtrate thus obtained was concentrated at 40°C with a rotary evaporator. A portion (5.0g) of the

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concentrated methanol extract was fractionated by the modified Kupchan partitioning protocol (Vanwagenen *et al*., 1993) which afforded pet-ether (1.0g), carbon tetrachloride (1.1g), dichloromethane (850mg) and aqueous (1.65g) soluble materials.

An aliquot of the dichloromethane soluble partitionate (650mg) was fractionated by column chromatography (CC) over silica gel (Kieselgel 60, mesh 70-230) using petroleum ether and ethyl acetate mixture in order of increasing polarities. A total of 143 fractions were collected, each 20ml. Preparative thin layer chromatography (PTLC) of column fractions eluted with 10% ethyl acetate in petroleum ether over silica gel using toluene - ethyl acetate (95:5) afforded compound **1** (3.5mg) and the yield value was 0.07%. Again, PTLC of column fractions 91 to 96 eluted with 50% ethyl acetate in petroleum ether over silica gel using 2% methanol in dichloromethane as the developing solvent gave compound **2** (7.0mg), **3** (6.5mg) having the yield value 0.14% and 0.13% respectively.

Results

Repeated chromatographic separation and purification of the carbon tetrachloride soluble partitionate of a methanol extract of the rhizomes of *C. longa* provided three compounds, the structures of which were determined by analysis of ^{1}H NMR and GCMS spectral as well as by comparison with previously reported values.

Turmerone (**1**): yellowish oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09 (4H, s, H-2, H-3, H-5, H-6), 6.06 (1H, s, H-12), 3.28 (1H, m, H-8), 2.70 (1H, dd, *J* = 15.6, 6.4 Hz, H_a-10), 2.59 (1H, dd, *J* = 15.6, 8.4 Hz, H_b-10), 2.29 (1H, s, H-7), 2.09 (1H, s, H_3-14), 1.84 (1H, s, H_3-15), 1.24 (1H, s, H_3-9); GCMS: *m/z* 216 appropriate for $C_{15}H_{20}O$.

***trans*-p-coumaric acid** (**2**): yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (1H, d, *J* = 16.0 Hz, H-7), 7.38 (2H, d, *J* = 8.0 Hz, H-2 & H-6), 6.78 (2H, d, *J* = 8.0 Hz, H-3 & H-5), 6.42 (1H, d, *J* = 16.0 Hz, H-8), 5.72 (1H, br. s, H-4).

***trans*-ferulic acid** (**3**)**:** yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (1H, d, *J* = 16.0 Hz, H-7), 7.09 (1H, d, *J* = 8.0 Hz, H-6), 7.04 (1H, br. s, H-2), 6.92 (1H, d, *J* = 8.0 Hz, H-5), 6.45 (1H, d, *J* = 16.0 Hz, H-8), 5.79 (1H, br. s, H-4), 3.94 (1H, br. s, H-3).

Discussion

The ^{1}H NMR spectrum (400 Hz, $CDCl_3$) of compound **1** (Figure 1) was almost identical to that acquired for turmeronol-A (**4**) (Imai *et al*., 1990) suggesting a close structural similarity between these two compounds. However, the ^{1}H NMR spectrum of compound **1** displayed a signal that integrated for four protons in the aromatic region at δ 7.09, instead of three resonances at δ 6.65 (H-3), δ 6.69 (H-5), and δ 7.01 (H-6) as seen in the spectrum of turmeronol-A. This clearly revealed that this aromatic ring in compound **1** was disubstituted as compared to a tetrasubstituted aromatic ring in **4** (Figure 1)**.** Thus, the signal at δ 7.09 could be assigned to H-2, H-3, H-5 and H-6. The ^{1}H NMR spectrum of **1** exhibited two double doublets centered at δ 2.58 (1H, *J* = 15.7, 6.0 Hz) and δ 2.68 (1H, *J* = 15.7, 8.0 Hz) which could be attributed to the geminal methylene protons at C-10. A singlet for an olefinic proton observed at δ 6.06 could be ascribed to H-12, while the multiplet of one proton intensity at δ 3.29 was assigned to the methine proton, H-8.

The spectrum also displayed three methyl signals at δ 2.09 (br. s), 1.84 (br. s) and 2.29 (br. s), in addition to a three proton doublet (*J* = 6.5 Hz) at δ 1.24. These signals were ascribed to the vinylic methyls (Me_2-13), aromatic methyl at C-1 (δ 2.29) and another methyl group at C-8, respectively. Therefore, the structure of compound **1** was deduced as turmerone by analysis of its ^{1}H NMR and GCMS data as well as by comparing with published values (Li *et al*., 2004).

The ^{1}H NMR spectrum of compound **2** (Figure 2) displayed two doublets (*J* = 8.4 Hz) centered at δ 6.78 and 7.38, each integrating for two protons each. Two down field doublets at δ 7.53 (1H, *J* = 16.0 Hz) and δ 6.42 (1H, *J* = 16.0 Hz) revealed the presence of a pair of *trans* coupled olefinic protons at H-7 and H-8 respectively. The spectrum also showed a broad singlet at δ 5.72 demonstrative of a hydroxyl group. The splitting pattern and coupling constants of the aromatic protons indicated the presence of 1,4 - disubstituted benzene ring. The above spectral features are in close agreement to those observed for *trans*-p-coumaric acid (Hussain *et al*., 2008). On the basis, compound **2** was characterized as *trans*-p-coumaric acid**.**

The ^{1}H NMR spectrum acquired for compound **3** (Figure 2) was almost identical to that of *trans*-p-coumaric acid (**2**), suggesting a close structural similarity between these two compounds. The ^{1}H NMR spectrum of compound **3** displayed a singlet of three proton intensity at δ 3.94 demonstrative of the presence of a methoxyl group at C-3. It also displayed a broad singlet at δ 7.04 (H-2) and two doublets (J = 8.0 Hz) centered at δ 6.92 (H-5) and 7.09 (H-6), each integrating for one proton, typical for a 1,3,4-trisubstituted aromatic moiety in compound **3**. The doublets (J = 16.0 Hz) centered at δ 7.50 and 6.45 could be assigned to the trans coupled protons H-7 and H-8, respectively. The relatively low field resonance of H-7 could easily be explained by its beta position to the carbonyl group, in the form of a carboxylic acid. On the basis, the compound **3** was characterized as *trans*-ferulic acid (**3**). The identity of compound **3** was further confirmed by comparison of its spectral data with published literature (Hussain *et al*., 2008).

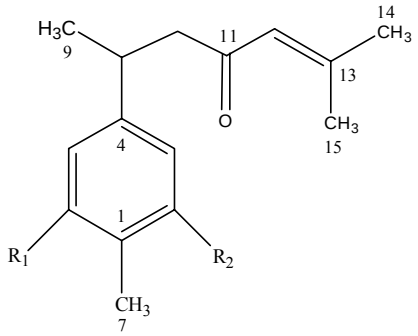

Figure 1: Compound 1 (Turmerone) (R_1 = R_2 = -H) Compound 4 (Turmeronol-A) (R_1 = R_2 = -OH)

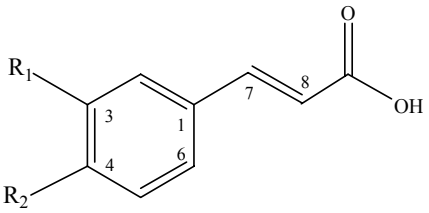

Figure 2: Compound 2 (*trans*-p-coumaric acid) (R_1 = -H; R_2 = -OH) Compound 3 (*trans*-ferulic acid) (R_1 = -OCH_3; R_2 = -OH)

Conclusion

The present phytochemical study of the carbon tetrachloride soluble fraction of the methanol extract of *C. longa* afforded a sesquiterpene and two phenylpropanoid derivatives, the structure of which were established as turmerone (**1**), *trans*-p-coumaric acid (**2**) and *trans*-ferulic acid (**3**) by extensive spectroscopic studies as well as by comparison with published result.

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