Facile isolation of \((E)-\text{labda-8(17),12-diene-15,16-dial}\) from \textit{Curcuma amada} and its conversion to other biologically active compounds

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Received 22 April 2013; accepted (revised) 20 September 2013

\textit{Curcuma amada} Roxb. (Zingiberaceae) rhizomes have been found to be a good source of \((E)-\text{labda-8(17),12-diene-15,16-dial}\). This has been chemically transformed to other biologically active compounds like aftramodial, zerumin A as well as other natural products like \((E)-\text{labda-8(17),12-diene-15,16-olide}\), \(15,16\)-epoxy-8(17),13(16),14-labdatriene and \((-)\)-marginatone. The antimicrobial activity of zerumin A sodium salt as well as other derivatives of the dialdehyde has been established.

**Keywords**: \textit{Curcuma amada}, zerumin A, \((E)-\text{labda-8(17),12-diene-15,16-dial}\), aftramodial, antimicrobial, chemical transformation.

Natural products play a significant role in the drug discovery process. Ethnobotanic leads have been pivotal to the development of many drugs which are used in the treatment of cancer as well as many other ailments. Plants belonging to \textit{Zingiberaceae} family have been used around the world extensively in medicinal preparations and as spices. Among them, the most renowned are \textit{Zingiber officinale} and \textit{Curcuma longa} which have provided many biologically active compounds like the gingerols and curcumin. However, even the lesser known members of this family like \textit{Curcuma zedoaria}, \textit{Curcuma aromatica}, \textit{Curcuma amada}, \textit{Kaempferia galanga}, \textit{Kaempferia rotunda}, \textit{Alpinia galanga}, \textit{Zingiber zerumbet}, etc. also are used in traditional medicinal preparations of India, China and many South East Asian countries. Several compounds having antioxidant as well as other useful biological activities have been isolated from these plants. Of these, \textit{Curcuma amada} Roxb. is cultivated extensively in South India, especially in Kerala, for use as a culinary spice. The plant is called ‘mango ginger’ since the rhizomes have the smell of fresh mango. In addition to a sesquiterpene dimer ‘difurocumenol’\textsuperscript{11} and a substituted sesquiterpene ‘amadannulen’\textsuperscript{2}, six diterpenoids have been isolated from the rhizomes of \textit{C. amada}, of which \((E)-\text{labda-8(17),12-diene-15,16-dial}\) \textbf{1} is the major component\textsuperscript{3,4}.

The dialdehyde \textbf{1} itself is reported to have antifungal activity against several \textit{Candida} species, mosquitocidal activity on \textit{Aedes aegyptii} larvae and cytotoxicity against KB cells\textsuperscript{5}. The column chromatographic purification of the crude extract of \textit{Curcuma amada} rhizomes led to the isolation of substantial amount of the dialdehyde, \textit{viz}, \((E)-\text{labda-8(17),12-diene-15,16-dial}\) \textbf{1}, in 6.9 g yield (1.7\% of dry weight of rhizomes). Isolation of this dialdehyde \textbf{1} bearing two differently functionisable aldehydic groups as well as the labdane ring structure with an exocyclic double bond from \textit{Curcuma amada} in good yield suggested the possibility of chemically transforming it to other novel biologically active compounds, thus amplifying its synthetic utility. Accordingly, it has been shown that the natural products aftramodial\textsuperscript{6} \textbf{2}, zerumin \textit{A}\textsuperscript{7} \textbf{3}, \((E)-\text{labda-8(17),13-diene-15,16-olide}\) \textbf{8} as well as the furanolabdane named earlier as 15,16-epoxy-8(17),13(16),14-labdatriene\textsuperscript{9} \textbf{8} can be synthesized readily from \textbf{1}. Compound \textbf{8} is also an advanced intermediate \textit{en route} to the natural product \((-)\)-marginatone\textsuperscript{10} \textbf{9}.

Aframodial \textsuperscript{8} \(\beta,17\)-epoxy-12(\(E\))-labdene-15, 16-dial \textbf{2} isolated from \textit{Aframomum aulacocarpus} possesses strong antifungal and antimicrobial activity\textsuperscript{6}. It also possesses strong cytotoxicity towards L1210 cells and KB cells\textsuperscript{11}. In another study, its
anti-hypercholesterolemic effect has also been demonstrated\textsuperscript{12}. The compound was also isolated from *Zingiber mioga* and confirmed as its pungent principle with the name Miogadial\textsuperscript{13}. Zerumin A 3 reported earlier from *Alpinia zerumbet* has been reported to be weakly active in the Sc-7 yeast strain assay for cytotoxicity\textsuperscript{14}. \(\left(\text{E}\right)\)-Labda-8(17),13-diene-15,16-olide 6 and \(8\beta,17\)-epoxylabd-13-ene-15,16-olide 10 have also been synthesized from compound 1 and preliminary tests confirmed that compounds 3, 6 and 10 have antimicrobial activity.

**Results and Discussion**

**Isolation of compound 1 and its chemical transformations**

\(\left(\text{E}\right)\)-Labda-8(17),12-diene-15,16-dial 1 was isolated through extraction of the dry rhizomes of *Curcuma amada* with chloroform and separation of the crude extract by column chromatography over silica gel. The structure was confirmed by comparison of spectroscopic data with those in the literature\textsuperscript{15}. The dialdehyde 1 was converted to aframodial 2 in 40% yield in a single step, \(\text{viz.}\), epoxidation using \textit{m}-chloroperbenzoic acid and the structure was confirmed by comparison of spectral data reported earlier\textsuperscript{6} (Figure 1).

On subjecting compound 1 to Jone’s oxidation conditions using \(\text{CrO}_3\) and \(\text{H}_2\text{SO}_4\), one of the aldehydic groups was found to oxidize readily leading to the acid, \(\text{viz.}\), Zerumin A, 3 (Ref 7).

Reduction of compound 3 using \(\text{NaBH}_4\) in methanol afforded compound 4 in good yield. Cyclisation of compound 4 using \(\text{p}\)-toluenesulphonic acid led to the formation of lactone 5 whose structure was confirmed from the spectral data. Rearrangement of lactone 5 using catalytic amount of 1,8-diazabicyclo[5.4.0]undecene\textsuperscript{16} in dichloromethane resulted in rearranged lactone, \(\text{viz.}\), \(\left(\text{E}\right)\)-labda-8(17),13-diene-15,16-olide 6. The rearranged lactone 6 was reduced with \(\text{LiAlH}_4\) to afford the compound 7 \(\text{viz.}\), \(\left(\text{E}\right)\)-labda-8(17),13(12)-diene-15,16-diol in 49% yield which was identified based on all these data and on comparison with those reported on literature\textsuperscript{9}.

Oxidation of the olefinic diol 7 thus obtained with pyridinium chlorochromate in dry dichloromethane in the presence of molecular sieves led to the cyclised furan compound 8 in 53% yield whose data matched for 15,16-epoxy-8(17),13(16),14-labdatriene 8 reported in literature\textsuperscript{9,10} from the marine sponge *Cacospongia* sp. thus confirming the structure.

The furanolabdane 8 which has been synthesized earlier by Kolympadi et al. through reduction of (+)-coronarin E has been further transformed into the marine furanoditerpene \(\text{viz.}\), (-)-marginatone 9 known to occur in the marine sponge *Aplysilla glacialis*\textsuperscript{10}. Therefore, the synthesis of 8 represents a formal total synthesis of (-)-marginatone 9.

The epoxidation of compound 6 was carried out using \textit{m}-chloroperbenzoic acid in dichloromethane to afford compound 10 (Figure 2).
The chemical transformations of \((E)\)-labda-8(17),12-diene-15,16-dial 1 are shown in Scheme I.

### Antimicrobial activity studies

The compounds zerumin A 3 as its sodium salt, \((E)\)-labda-8(17),13-diene-15,16-olide 6 and \(8\beta\), 17-epoxylabd-13-ene-15,16-olide 10 were tested for antimicrobial activity\(^{17}\) with \textit{Escherichia coli} as the test organism. \textit{Escherichia coli} is one of the main species of bacteria living in the lower intestine of mammals. \textit{Gentamicin}, which is an aminoglycoside antibiotic and can treat many types of bacterial infections, was used as the control. Different concentrations of the compounds in dimethylformamide were taken. On measuring the diameter of the zone of inhibition, it was found that all these compounds exhibit significant biological activity. Of the three compounds chosen for the study, the most efficient antimicrobial agent was found to be compound 3 which showed significant activity even at 20 \(\mu\)g concentration. The antimicrobial activity was dose dependant as evident from the Table I.

The biologically active compound 1 could thus be transformed to various other natural products of interest. In addition, the antimicrobial activity of zerumin A 3, the lactone 6 and the epoxide 10 has been established from the preliminary studies conducted on \textit{E. coli}. Thus the rhizomes of \textit{Curcuma amada} can therefore be considered as a good resource for biologically active compounds.

### Experimental Section

All melting points are uncorrected and were determined on a Meltemp II hot stage melting point apparatus. The IR spectra were recorded on Shimadzu-FTIR spectrometer. \(^1\)H and \(^{13}\)C NMR spectra were recorded at 300 MHz and 75 MHz respectively using deuterated solvents on Bruker DPX 300 MHz FT NMR spectrometer. Tetramethylsilane was used as internal standard and chemical shifts are expressed on the \(\delta\)-scale. Mass spectra were recorded using Jeol JMS 600H mass spectrometer.

Analytical thin layer chromatography was performed on Merck silica gel 60 F\(_{254}\) aluminium sheets. The spots were first checked under UV light and later in an iodine chamber also. The TLC plates were kept in an air oven for 20 min at 80\(^{\circ}\)C prior to use. Column chromatography was carried out with 100-200 mesh silica gel. All the solvents used for chromatography were of commercial grade and were purified prior to use. The solvents were removed under reduced pressure using Büchi rotary evaporator.

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**Table I — Zone of inhibition of microbial growth**

<table>
<thead>
<tr>
<th>Disc concentration</th>
<th>Inhibition zone diameter (mm)*</th>
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<tbody>
<tr>
<td></td>
<td>Compd 3</td>
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<tr>
<td>Gentamicin (10 mg)</td>
<td>30</td>
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<td>DMF</td>
<td>10</td>
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<tr>
<td>20 (\mu)g</td>
<td>20</td>
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<tr>
<td>80 (\mu)g</td>
<td>25</td>
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<tr>
<td>160 (\mu)g</td>
<td>28</td>
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<tr>
<td>200 (\mu)g</td>
<td>30</td>
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</table>

\*\(n=2\), values are mean zone of inhibition
Drying of the plant material was carried out in RRLT-NC drier.

The reactions requiring dry conditions were carried out under nitrogen or argon atmosphere in solvents which were dried according to literature procedures. Extraction of reaction mixtures were carried out with appropriate organic solvents, with repetition using fresh solvent before the organic layers were combined. Washing of the organic layer was also repeated three times in each case (distilled water, saturated sodium bicarbonate solution, brine, etc.) as required by the procedure.

Isolation of compound 1

Three kilograms of *Curcuma amada* rhizome was collected from Kottayam District, Kerala. It was then thoroughly washed, chopped, dried and powdered to obtain 400 g of powdered material. The powdered material (400 g) was subjected to cold extraction using chloroform. Removal of chloroform under reduced pressure gave 23 g of crude extract. About 500 g of silica gel was packed in a 1000 mL glass column using petroleum ether as solvent and 23 g of crude extract was loaded and eluted initially with petroleum ether. Further, polarity was gradually increased using petroleum ether-ethyl acetate mixtures and a total of 182 fractions of 100 mL each were collected. According to the similarities in TLC, they were pooled together to get 19 fraction pools. Fifth fraction pool (fractions 36 to 43) which was obtained upon elution with 10% ethyl acetate in petroleum ether showed one major compound in TLC. It was repurified with 2% ethyl acetate in petroleum ether over silica gel column which afforded the compound 1 in 6.9 g yield. The structure of the compound was confirmed by $^1$H and $^{13}$C NMR, IR, and mass spectroscopic studies.

Chemical transformations of compound 1

Synthesis of aframodial, 2

To an ice-cooled solution of the compound 1 (0.060 g, 0.22 mmol) in dichloromethane, $m$-chloroperbenzoic acid (0.070 g, 0.404 mmol) was added over 1 hr time and the reaction mixture was allowed to attain RT. After completion of the reaction as indicated by thin layer chromatography, sodium thiosulphate crystals were added and the reaction mixture was stirred for an additional 30 min to break the excess $m$-chloroperbenzoic acid. The reaction mixture was filtered, extracted with dichloromethane (3 × 20 mL) washed with saturated sodium bicarbonate solution, distilled water and brine. The solvent was removed and the crude compound on purification by column chromatography with 5% ethyl acetate in petroleum ether yielded aframodial 2 in 40% yield. The IR, and $^1$H and $^{13}$C NMR matched with that reported in literature.

Synthesis of zerumin A, 3

To a stirred solution of compound 1 (0.500 g, 1.7 mmol) in acetone, Jone’s reagent was added until the reddish colour persisted. After completion of reaction as indicated by thin layer chromatography, excess reagent was quenched by the addition of few drops of isopropanol. The reaction mixture was then filtered through celite, concentrated, further extracted with ether (3 × 25 mL), washed with distilled water, brine and dried with anhydrous Na$_2$SO$_4$. Solvent was removed and the residue was subjected to chromatography with 10% ethyl acetate in petroleum ether over silica gel column which afforded the zerumin A 3 in 60% yield, 0.313 g. The IR, and $^1$H and $^{13}$C NMR matched with that reported in literature.

Synthesis of (E)-labda-8(17),12-diene-16-ol-15-oic acid, 4

To an ice-cooled solution of compound 3 (0.960 g, 3.0 mmol) in dry methanol, NaBH$_4$ (0.223 g, 6.03 mmol) was added over 5 min time and the reaction was allowed to attain RT gradually. The reaction was then quenched with cold water, the methanol removed under reduced pressure and the product extracted with dichloromethane (3 × 25 mL). Then the solvent was removed and the residue purified by column chromatography with 15% ethyl acetate in petroleum ether to afford compound 4 in 98% yield, 0.940 g.

FT-IR (CH$_2$Cl$_2$): 3400, 1710, 1149, 890 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 6.08 (br s), 5.59 (1H, t, $J$ = 6 Hz), 4.81 (1H, s), 4.43 (1H, s), 4.09 (2H, s), 3.22 (2H, s), 2.50-2.25 (2H, m), 2.10-1.90 (3H, m), 1.85-1.60 (4H, m), 1.60-0.95 (6H, m), 0.80 (3H, s). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 176.1, 148.3, 133.6, 129.9, 107.4, 72.5, 56.8, 55.3, 42.0, 39.4, 39.1, 37.9, 33.6, 33.5, 29.6, 24.1, 22.5, 21.7, 19.3, 14.3; LRMS, EI [M-18]$^+$: m/z 302.03. C$_{20}$H$_{32}$O$_2$ requires 320.24.

Synthesis of (E)-labda-8(17),12-diene-15,16-olide, 5

To a solution of compound 4 (0.350 g, 1.08 mmol) taken in dry dichloromethane, catalytic amount of p-toluenesulphonic acid was added and stirred at RT
for 20 hr. At the end of this period, the mixture was filtered, extracted with dichloromethane (3 x 20 mL), washed with distilled water, brine, dried with anhydrous Na₂SO₄ and then concentrated. The concentrate thus obtained was subjected to chromatography with 5% ethyl acetate in petroleum ether over silica gel column to obtain compound 5 in 92% yield, 0.300 g.

FT-IR (CH₂Cl₂): 2928, 1784, 1643, 1459, 1387, 1171, 1017, 887 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.43 (1H, m), 4.83 (1H, s), 4.79 (2H, s), 4.42 (1H, s), 3.15 (2H, s), 2.45-2.35 (1H, m), 2.35-2.15 (1H, m), 2.15-1.85 (2H, m), 1.85-1.65 (4H, m), 1.65-1.0 (6H, m), 0.88 (3H, s), 0.82 (3H, s), 0.71 (3H, s); ¹³C NMR (75 MHz, CDCl₃): δ 175.7, 148.3, 127.8, 125.2, 107.1, 72.3, 56.7, 55.3, 42.0, 39.4, 39.1, 37.9, 33.5, 33.4, 29.6, 24.2, 24.1, 21.6, 19.3, 14.3; HRMS (EI) [M⁺] requires 306.26; [α]D²⁶: +38.3° (c 0.09, CHCl₃).

Synthesis of (E)-labda-8(17),13-diene-15,16-olide, 6
To a solution of compound 5 (0.040 g, 0.14 mmol) in dry dichloromethane, a catalytic amount of 1,8-diazabicyclo[5.4.0]undecene was added and stirred at RT for 30 min. Then the mixture was filtered, extracted with dichloromethane (3 x 10 mL) and worked up as given in the general procedure. The crude residue was purified by chromatography over silica gel column with 5% ethyl acetate in petroleum ether to afford the product 6 in 85% yield, 0.035 g. The IR, and ¹H and ¹³C NMR matched with that reported in literature⁸,¹⁸,¹⁹.

Synthesis of (E)-labda-8(17),13-diene-15,16-diol, 7
To a suspension of LiAlH₄ (0.098 g, 4 eq) in dry tetrahydrofuran was added dropwise anhydrous AlCl₃ (0.104 g, 1.2 eq) in tetrahydrofuran at 0°C. Compound 6 (0.195 g) in dry tetrahydrofuran was then added and this mixture was stirred until completion of the reaction as indicated in thin layer chromatography. It was then extracted with ether and concentrated. The crude product was chromatographed over silica gel with 15% ethyl acetate in petroleum ether to afford diol 7 in 49% yield, 0.101 g.

m.p. 118-120°C; FT-IR (CH₂Cl₂): 3318, 2924, 1643, 1440, 1401, 1382, 1015, 883 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.60 (1H, t, J = 6.9 Hz), 4.84 (1H, s), 4.52 (1H, s), 4.19 (3H, d, 6.9 Hz), 4.15 (1H, d, J = 3 Hz), 2.41-2.29 (4H, m), 2.01-1.82 (2H, m), 1.75-1.05 (12H, m), 0.87 (3H, s), 0.80 (3H, s), 0.68 (3H, s); ¹³C NMR (75 MHz, CDCl₃): δ 148.5, 143.6, 125.8, 106.1, 59.8, 57.8, 56.3, 55.4, 42.0, 39.7, 39.0, 38.9, 34.2, 33.4, 33.4, 24.3, 22.0, 21.6, 19.2, 14.3; LRMS, FAB [M+23]⁺: m/z 329.01. C₂₀H₃₉O₂ requires 306.26; [α]D⁰: +38.3° (c 0.09, CHCl₃).

Synthesis of furanolabdane, 8
To a stirred solution of compound 7 (0.040 g) in dry dichloromethane, a solution of pyridinium chlorochromate (0.084 g, 3 eq) in dry dichloromethane was added drop-wise at RT under argon atmosphere. Then, the mixture was poured into cold water and the organic phase was separated and the aqueous phase was extracted with dichloromethane and usual work up was carried out. The residue was purified by silica gel column chromatography with petroleum ether to afford the product 8 as colourless oil in 53% yield, 0.020 g. The IR, and ¹H and ¹³C NMR matched with that reported in literature⁹.

Synthesis of 8β,17-epoxylabd-13-ene-15,16-olide, 10
To an ice-cooled solution of the compound 6 (0.050 g, 0.17 mmol) in dichloromethane, m-chloroperoxybenzoic acid (0.057 g, 0.33 mmol) was added over 1 hr time and the reaction mixture was stirred and allowed to attain RT. After completion of the reaction as indicated by TLC, sodium thiosulphate crystals were added and stirred for 30 min to break the excess m-chloroperoxybenzoic acid. The reaction mixture was filtered, extracted with dichloromethane (3 x 10 mL) as per general work up procedure and the crude product was purified by column chromatography with petroleum ether. The product 10 was obtained in 50% yield, 0.025 g.

m.p. 86-88°C; FT-IR (CH₂Cl₂): 3318, 2924, 1748, 1635, 1454, 1166, 1127, 1015 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.84 (1H, t, J = 1.6 Hz), 4.72 (2H, ABq, J = 17.4 Hz), 2.75 (1H, m), 2.54 (2H, d, J = 6 Hz), 2.43-2.29 (1H, m), 1.95-1.81 (2H, m), 1.73-1.40 (7H, m), 1.25-1.00 (5H, m), 0.91 (3H, s), 0.84 (3H, s), 0.83 (3H, s); ¹³C NMR (75 MHz, CDCl₃): δ 174.1, 170.7, 115.0, 72.9, 58.8, 54.8, 53.1, 50.6, 41.6, 40.1, 39.0, 36.2, 33.3, 33.2, 29.8, 21.6, 21.4, 19.5, 18.4, 14.5; LRMS, FAB [M+1]⁺: m/z 319.28. C₂₀H₃₉O₂ requires 318.22.

Antimicrobial screening
Gentamicin was used as the control with Escherichia coli as the test microorganism.
Antimicrobial activity was determined by agar well diffusion method\(^\text{17}\). 5 mg each of compounds 3, 6 and 10 were diluted to 1 mL with DMF. Concentrations of 20 µg, 80 µg, 160 µg and 200 µg concentrations from each were used for the study. Pure DMF was also taken as another control. The experiment was performed in duplicate. Microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values are presented in Table I.

Conclusion

In conclusion, herein is described a method for obtaining the biologically active compound \((E)\)-labda-8(17),12-diene-15,16-dial 1 rapidly from Curcuma amada in large quantity. Further, the transformation of the above compound to several biologically active compounds such as aframodial 2 and zerumin A 3 as well as other natural products 6, 8 and 9 is described. The synthesis of furanolabdane 8 also constitutes a formal total synthesis of the marine natural product marginatone 9. Antimicrobial activity of several compounds obtained through the transformation is also established.

Acknowledgement

AS thanks CSIR, New Delhi for financial assistance in the form of Junior and Senior Research Fellowships.

References