

Research Article

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Isolation of (Z)-7-methoxy-1, 5-dihydrobenzo[c] oxepine from *Curcuma caesia* Roxb.

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Abstract

Isolation, purification and finally Chemical characterisation of (Z)-7-methoxy-1, 5-dihydrobenzo[c] oxepine was done describe in this paper. This compound seemed to be a terpenoid isolated from *Curcuma caesia* Roxb., an endemic and naturally occurring triploid plant of northeast Asia. For chemical characterization of the compound various spectroscopic techniques like UV, IR (FT-IR), HRMS and NMR was used. The compound was positive in Liebermann's Burchard test and had melting point of 570C. Best of our knowledge this was the first report of presence of (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine in plants.

Keywords: *Curcuma caesia* Roxb., (Z)-7-methoxy-1, 5-dihydrobenzo [c] oxepine, Physicochemical characterization

Introduction

Curcuma caesia Roxb. (Black turmeric) of the family Zingiberaceae is a natural triploid, endemic and ethnomedicinally important plant. This plant was used by the tribal's of northeast India for its unique medicinal properties. There was no such report on the bioactive potentialities of black turmeric. So far eight metabolites have been isolated and characterised from *Curcuma caesia* Roxb. like Borneol, Borneol acetate, 1,8-Cineole, α -Curcumene, γ -Curcumene, β -Elemene, (E)- β -Ocimene, ar-Turmerone etc.^{1, 2} Here we report for the first time the presence of (Z)-7-methoxy-1,5-dihydrobenzo[c] oxepine from the shade dried rhizome of *Curcuma caesia* Roxb. Best of our knowledge this compound has seem to be a novel one which was not reported earlier.

Materials and Methods

Collection of Plant Material

Whole plant of *C. caesia* Roxb. was collected in the month of July 2010 from experimental garden of Department of Botany, University of Kalyani, and was identified in the Department of Botany, University of Kalyani, Nadia.

Extraction and Isolation of Crude Secondary Metabolite Content

2.5 kg shade dried rhizomes of black turmeric plant was powdered of approximately and extracted three times with 1 liter of 95% EtOH at room temperature to give an extract of 479 gms. The extract was evaporated under reduced pressure and a solid

residual mass was obtained. The above obtained residual sample was subjected to repeated preparative thin layer chromatography using different solvent systems, e.g solvent system 1. Methanol (5%): benzene (95%) and solvent system 2. Chloroform (60%): benzene (30%): acetic acid (10%). Three homogeneous spots were collected in solvent system 2, having Rf values of 0.87, 0.79 and 0.75 respectively. The sample with Rf value 0.75 was taken up for further study. This sample was positive in Liebermann's Burchard test³ and gave purple colour indicating its terpenoid nature. The compound had melting point of 57°C. The sample was then further analysed through various spectroscopic techniques like UV spectroscopy (UV-1601PC, UV-Visible Spectrophotometer, Shimadzu), FT-IR spectroscopy (Perkin Elmer Spectrum-1 Spectrophotometer), High Resolution Mass spectroscopy (JEOL- JMS 600 Instrument) and Nuclear Magnetic Resonance spectroscopy, ¹H & ¹³C (Bruker Avance- 400 Spectrometer) for its proper physicochemical characterization.

UV Spectroscopy of the Isolated Sample

The methanolic spectrum of the sample showed λ max at 854.0 nm, 522.0 nm, 476.50 nm, 281.50 nm, 228.0 nm and absorbance at = 0.0010, 0.0035, 0.0036, 1.8965, 3.0211 respectively. (Spectrum 1)

Results and Discussions

Chemical Characterization of the Isolated Sample

The compound was reddish yellow amorphous solid and was readily soluble in methanol. The melting point of the sample was 57 °C (Rf- 0.75 in solvent system 2) and it turned purple in Liebermann's Burchard test.³ The TLC chromatogram of the compound showed single spot when visualized under exposure of UV light as well as iodine vapor.

Baeyer's Test for Presence of Double or Triple Bond

In ~2-3 mg of the isolated compound in methanol was added in the very much diluted alkaline solution of potassium permanganate. The purplish pink colour of the reaction mixture turns to brown indicating presence of an active unsaturation (double or triple bond) in the compound.

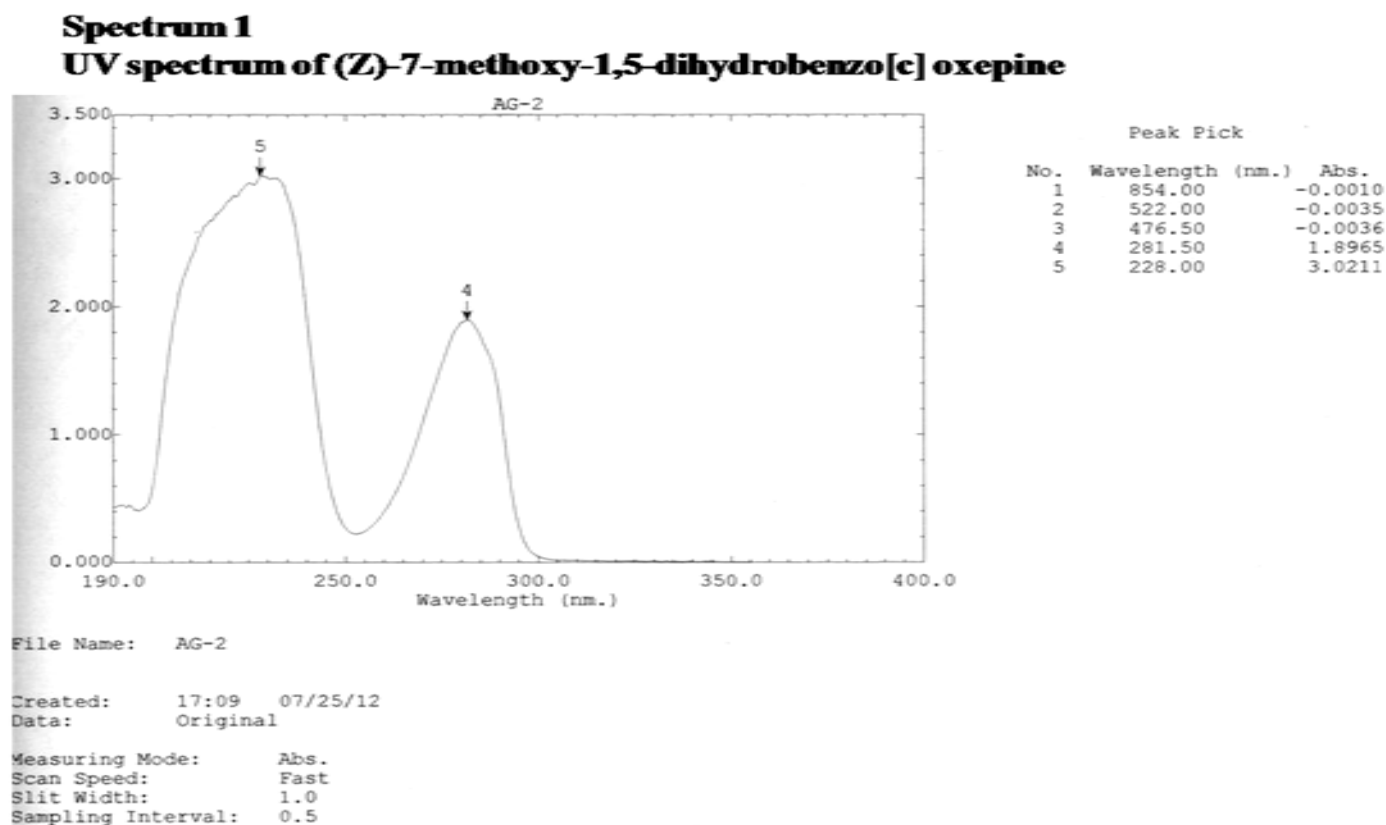


Figure 1: Spectrum 1

IR (FT-IR) Spectroscopy of the Isolated Sample

The IR spectrum of the sample showed ν (cm⁻¹): 3078, 2939, 2843, 1638, 1612, 1514, 1465, 1432, 1368, 1268, 1234, 1149, 1122, 1035, 996, 817, 793 and 747. (Spectrum 2)

Spectrum 2

IR (FT-IR) spectrum of (Z)-7-methoxy-1,5-dihydrobenzo[c]oxepine

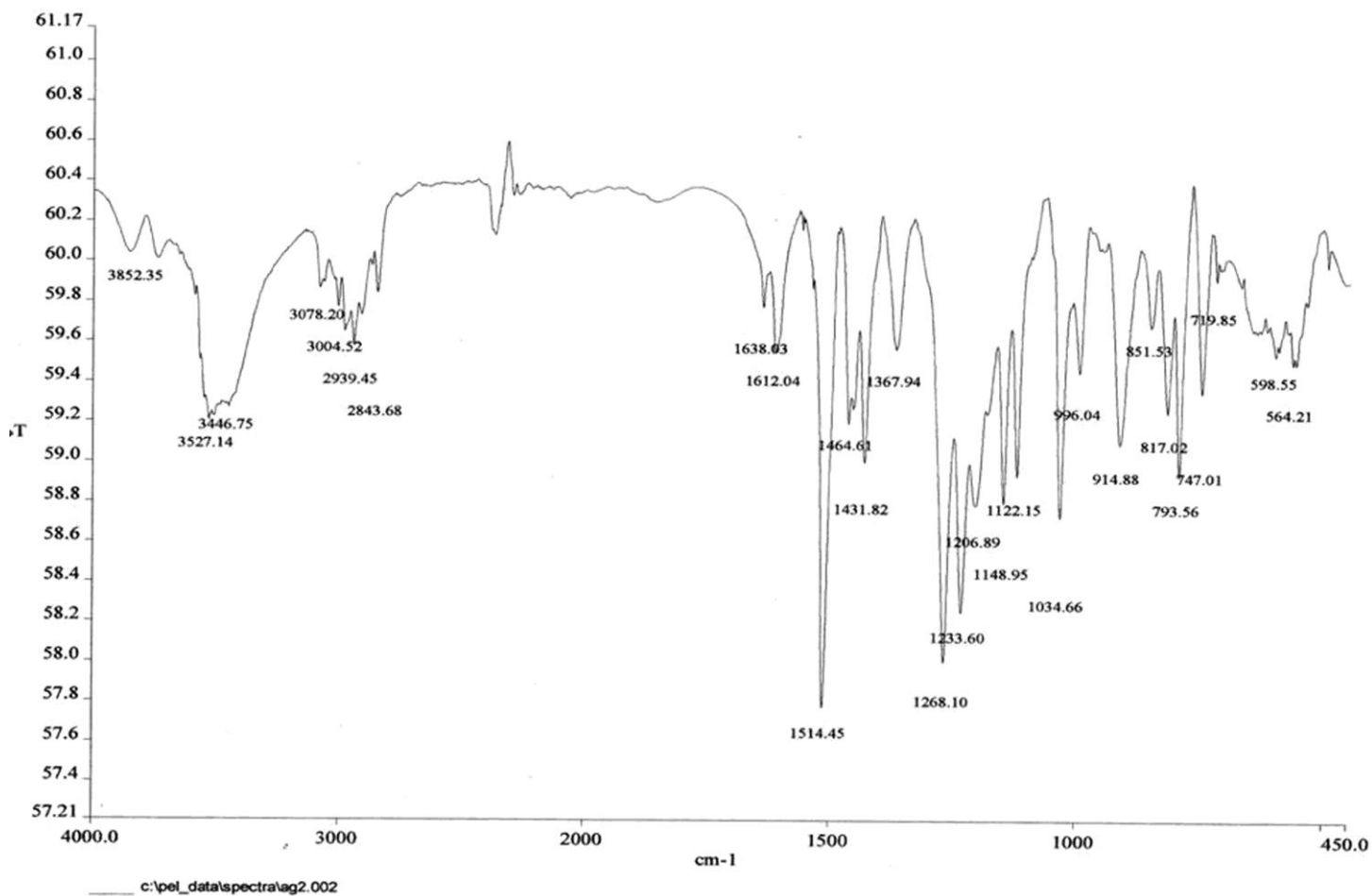


Figure 2: Spectrum 2

High Resolution Mass spectroscopy of the Isolated Sample

The mass of the sample was noted as to be (TOF MS ES⁺) 528. 9609 (3M⁺) (Spectrum 3)

Spectrum 3

HRMS spectrum of (Z)-7-methoxy-1,5-dihydrobenzo[c] oxepine (Arrow indicates trimeric mass (3 M⁺))

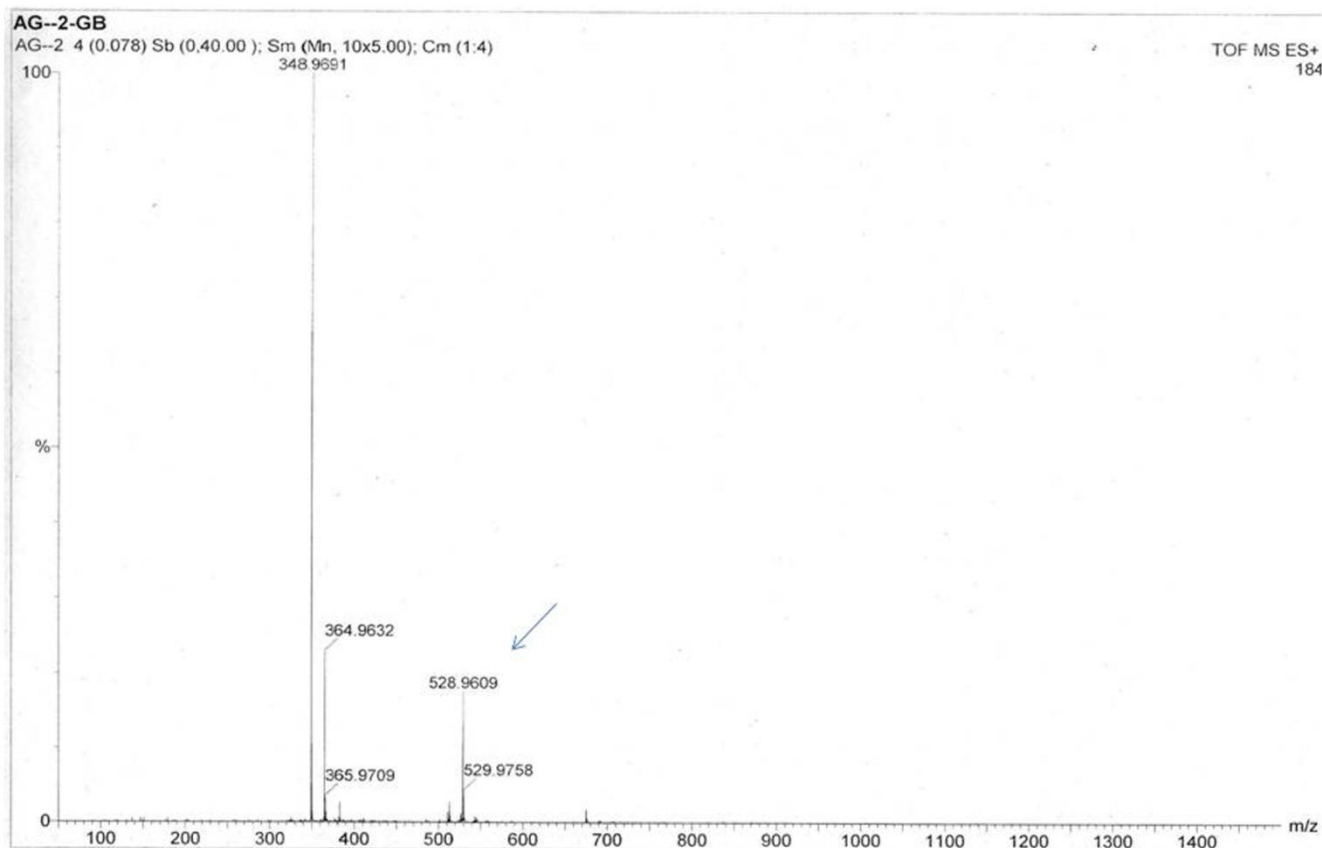


Figure 3: Spectrum 3

Nuclear Magnetic Resonance Spectroscopy of the Isolated Sample

¹H NMR (400 MHz, CDCl₃):

δ . 6.84 (1H, dd, J = 8.4, 3.6 Hz, Ar-H-8), 6.67 (2H, d, J = 5.2 Hz, Ar-H-9 and Ar-H-6), 5.95 (1H, dt, J = 8.4, 6.8 Hz olifinic-H-4), 5.63 (1H, s, olifinic-H-3), 5.08-5.03 (2H, m, H-1), 3.82 (3H, s, H-12), 3.30 (2H, d, J = 6.4 Hz, H-5). (Spectrum 4)

Spectrum 4

Proton NMR spectrum of (Z)-7-methoxy-1,5-dihydrobenzo[c]oxepine

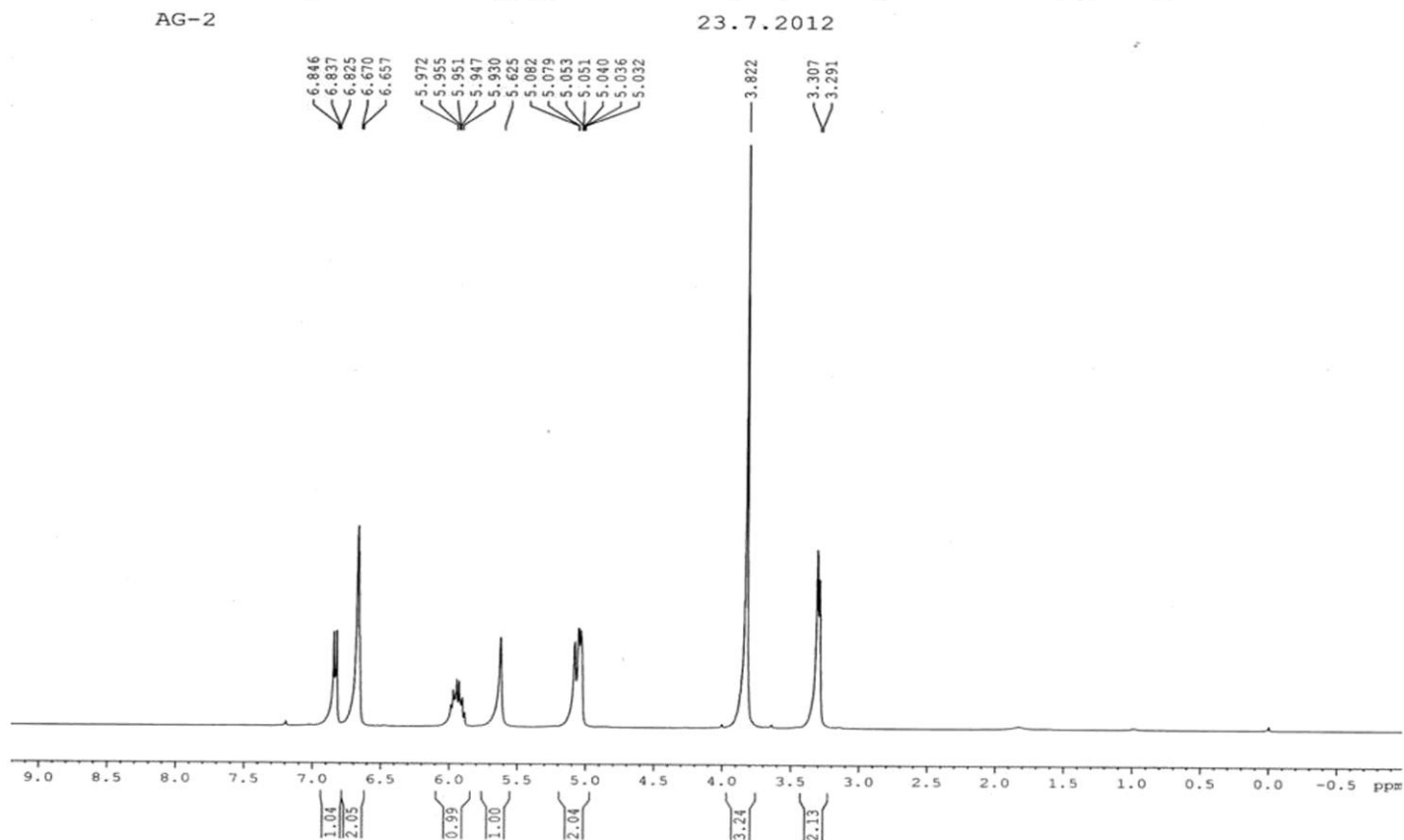


Figure 4: Spectrum 4

¹³C NMR and DEPT-135 (100 MHz, CDCl₃):

δ. 146.6 (C), 144.0 (C), 138.0 (CH), 132.0(C), 121.3 (2CH), 115.6 (CH₂), 114.5 (CH), 111.3 (CH), 55.9 (CH₃), 39.9 (CH₂). (Spectrum 5 & 6)

Spectrum 5

Carbon NMR spectrum of (Z)-7-methoxy-1,5-dihydrobenzo[c]oxepine

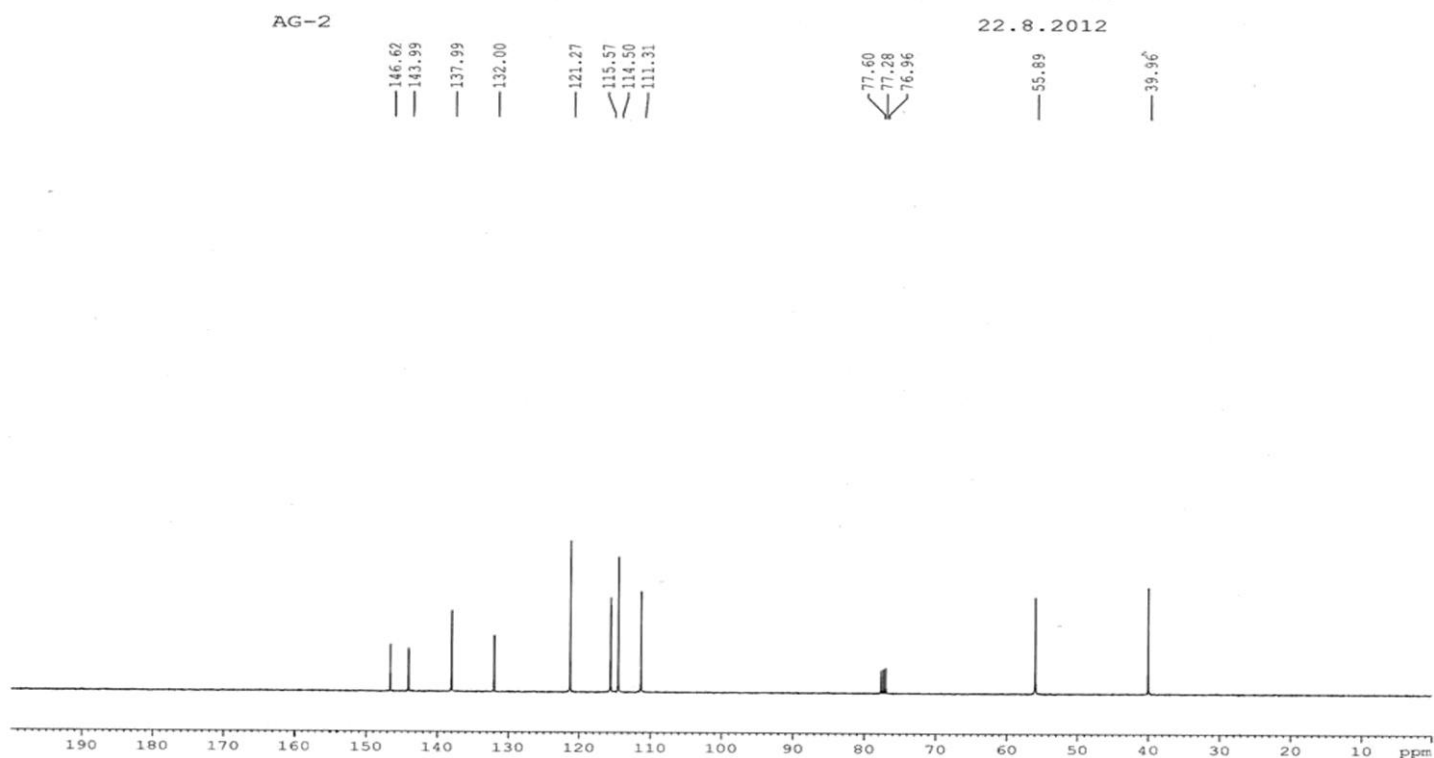


Figure 5: Spectrum 5

Spectrum 6

DEPT-135 spectrum of (Z)-7-methoxy-1,5-dihydrobenzo[c]oxepine

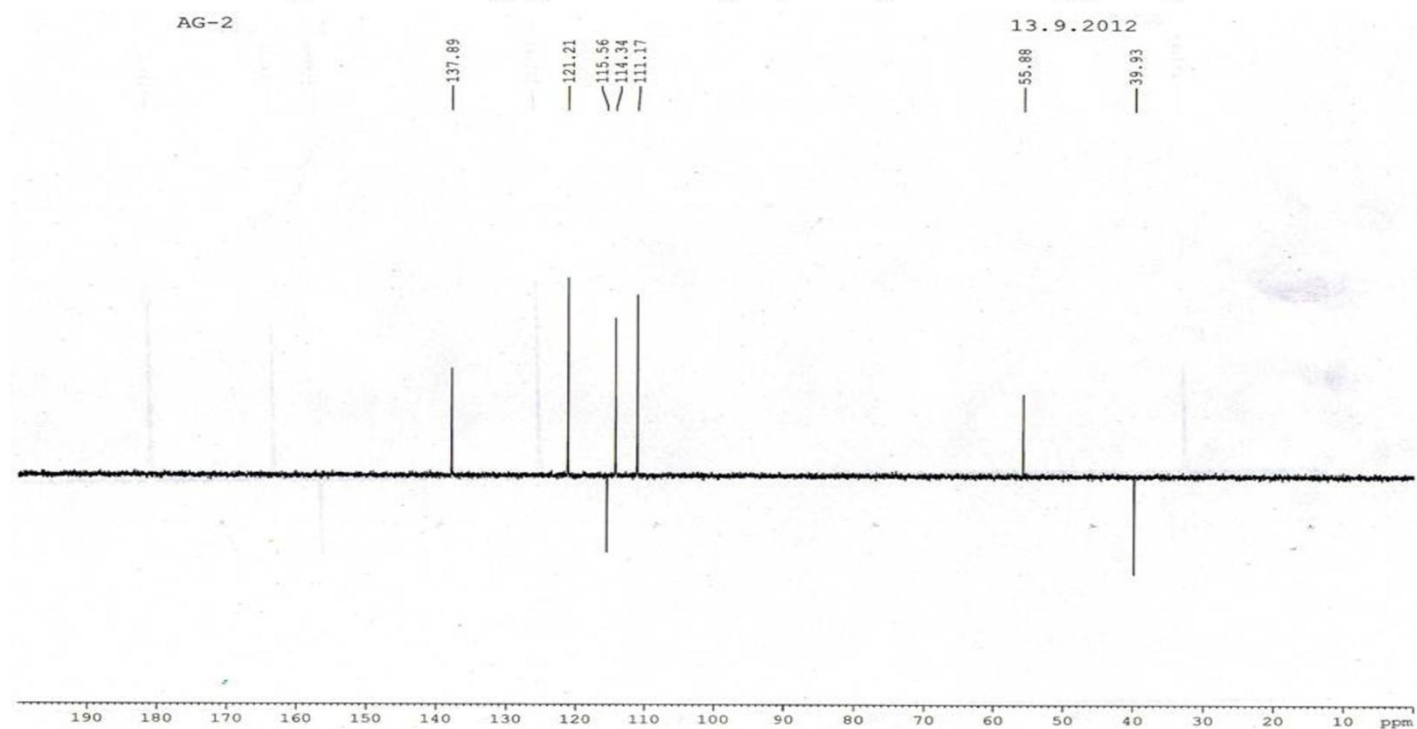


Figure 6: Spectrum 6

Interpretation of the Structure of the Isolated Compound

The U.V spectrum of the compound showed the intense absorption peak (λ max) at 281 nm, indicating the presence of benzene ring with auxochromic functionality. When I.R spectrum of this compound was recorded, no characteristic strong absorption peaks in functional group region were found. The weak absorption at 3078 cm^{-1} was assigned for aromatic C-H stretching. Aliphatic C-H stretching was also found at 2939 cm^{-1} (asymmetric) and 2843 cm^{-1} (symmetric). An absorption peak of medium intensity at 1612 cm^{-1} was also observed indicating the aliphatic C=C stretching. In addition to that, the quite intense band at 1514 cm^{-1} indicated the existence of aromatic C=C stretching.⁴

¹H-NMR spectrum of compound in figure 1 exhibited the presence of 12 protons. Among which $\delta = 6.84$ ppm (1H, dd, $J = 8.4, 3.6$ Hz, H-8) and 6.67 ppm (2H, d, $J = 5.2$ Hz, H-9 and H-6), confirmed the presence of three aromatic protons. Two olefinic protons had been obtained at $\delta = 5.95$ ppm (1H, dt, $J = 8.4, 6.8$ Hz, H-4) and at 5.63 ppm (1H, s, H-3). Two different type of -CH₂ (Methylene) protons was also observed at 5.08-5.03 ppm (2H, m, H-1), and 3.30 ppm (2H, d, $J = 6.4$ Hz, H-5). Remaining 3 protons of methyl group was located at 3.82 (3H, s, H-12) in the ¹H-NMR-spectrum.

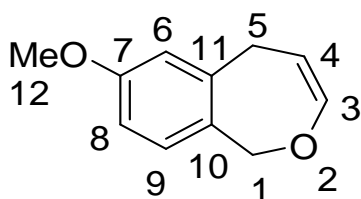


Figure 7: Structure of (Z)-7-methoxy-1,5-dihydrobenzo[c]oxepine

The ten peaks in ¹³C-NMR-spectrum clearly indicated the presence of 11 different carbon atoms in which one signal at $\delta = 121.3$ ppm was accounted for two signals with double intensity both in ¹³C-NMR and DEPT-135 spectra. The DEPT-135 spectrum of the compound in figure 7 showed 7 signals out of which two are negative signals ($\delta = 115.6$ and 39.9 ppm) indicating the presence of two methylene carbon in the compound. Remaining five positive signals were accounted for five methine ($\delta = 137.9, 121.3, 114.3, 111.2$ ppm) and one methyl carbons ($\delta = 55.9$). Thus based on the ¹³C-NMR and DEPT-135 spectral data, compound in figure 7 was accounted to have one methyl, two methylene, five methine and three

quaternary carbons. The HRMS-spectrum of the isolated compound was found 528.9609 ($3M^+$). Hence, the molecular formula of the isolated fraction must be C₁₁H₁₂O₂ and its structure was shown in Figure 7.

Acknowledgements

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