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Isolation and Identification of a Chalcone From *Baccopa monnieri*

A. Suresh, X. Queen Rosary Sheela, R. Kanmani, C. Mani,
Lalith Easwaran‡, A. Leo stanley† and V. Alex ramani*
Department of Chemistry, St. Joseph's College (Autonomous), Tiruchirappalli-620 002, India
E-mail: alex140760@yahoo.com

The phytochemical analysis of the plant *Bacopa monnierie* reveals that it contains a chalcone type of compound 2,4,6-trihydroxy-5-(3,3-dimethyl propenyl)-3-(4-hydroxyphenyl) propiophenone. This chalcone compound is extracted using ethyl acetate solvent and characterized by applying chemical and spectral methods.

Key Words: Phytochemical analysis, *Bacopa monnierie*, Chalcone, Trihydroxy propiophenone.

INTRODUCTION

Chalcones are naturally occurring compounds with phenolic and ketonic functionality. The medicinal plant[1,2] *Bacopa monnierie* is analyzed phytochemically to have a chalcone. Indian Ayurvedic system of medicine suggests that *Bacopa monnierie* is useful as emetic, laxative and as medicine in treating bad ulcers, tumours, ascites, enlargement of spleen, indigestion, inflammations, leprosy, anaemia and billousness. The Unani system of medicine, reveals the fact that it is good in scabies, leucoderma, syphilis, a promising blood purifier and useful in diarrhea and fevers. It is reported to improve the intellect and it is used in the indigenous systems of medicine for the treatment of asthma, hoarseness, insanity, epilepsy and as a potent nervetonic, cardio tonic and diuretic. The juice of the leaves is given to children for relief in bronchitis and diarrhea. The paste of the leaves is used as a remedy for rheumatism.

Therapeutic evaluation was done by Yadav *et al.*[3] anti-epileptic drug was reported by Smith[4] from this plant. Some curative effect of *Bacopa monnierie* to the Alzheimer's diseases was reported by Enz *et al.*[5]. Bacopa saponin E and F were isolated by Mahato *et al.*[6]. The memory enhancing property in *Bacopa monnierie* was reported by Bhattacharya and his co-workers[7]. Bacopaside I and II were isolated by Chakravarty *et al.*[8] and Bacopaside III, bacopa saponin G, bacopaside A, B and C were isolated from this plant by Hou *et al.*[9]. Bacopaside III-V (three new terpenoid glycosides) was reported by Chakravarthy *et al.*[10]. A triterpenoid

†Department of Chemistry, St. Joseph's College of Arts & Science, Cuddalore-607 001, India.
‡Department of Chemistry, MAM College of Engineering, Siruganur, Tiruchirappalli-621 105, India.

saponin was isolated by Mahato *et al.*[6]. Garai *et al.*[11] isolated Bacopa saponin D. Bacoside A-1, minor saponin and bacosides A2-A, A3-A were isolated by Kulshreshtha *et al.*[12].

EXPERIMENTAL

The shade-dried plant material of *Bacopa monnierie* is soxhlet extracted[13] using ethanol solvent. The ethanolic extract is concentrated further by distillation. The chlorophyll present in the concentrated extracts was removed by treating with 4 N dil. H_2SO_4 at 60 ºC on a water bath for 30-45 min and filtering it. The ethanolic extract was further extracted with chloroform and then with ethyl acetate[14].

The micro TLC is done using the plate (7.5 cm × 2.5 cm) coated with 100 micron silica gel (0.2 g/plate) as stationary phase and using suitable eluants. The compounds separated are noted down. The details of the micro TLC are given in the Table-1.

TABLE-1
THE DETAILS OF THE MICRO TLC OF THE EXTRACTS

S. no.	Extract	Eluant	No. of compounds separated
1.	Chloroform extract	C_6H_6 : $CHCl_3$: EtOAC 1 : 1 : 2	BMC_1, BMC_2, BMC_3, BMC_4, BMC_5 {5-components}
2.	Ethyl acetate extract	C_6H_6 : EtOAC 4 : 1	BME_1, BME_2, BMC_3, BME_4, BME_5, BME_6, {6-components}

The preparative TLC[15] is carried out using the plate (20 cm × 20 cm) coated with 100 micron silica gel (5 g/plate) and suitable eluant as given in the Table-1. The components separated as bands are isolated by extraction using acetone from the silica gel. The isolated components are purified by recrystallization using acetone. Of the several components, the BME_2 is taken for characterization as it is in large quantity (500 mg).

The solubility of the compound BME_2 (m.p. 260-262 ºC) was tested positively in solvents- acetone, ethanol, ethyl acetate and acetonitrile. BME_2 decolorized bromine-alcohol reagent indicating the presence of unsaturation. It showed a positive response with Borsche's reagent, by giving orange colouration indicated presence of keto group. On oxidation with alkaline potassium permanganate it gave two aromatic acids-2,4,6-trihydroxybenzoic acid and 4-hydroxybenzoic acid. The BME_2 reacted with neutral ferric chloride solution to give green-red colouration indicating the phenolic functionality[16].

The molecular mass of the substance was deciphered to be 341.56 by the cryoscopic method using camphor solvent[17]. The UV-VIS spectrum was taken on the spectrophotometer, Lamda 35 model using spectroscopic grade ethanol. The FT-IR spectrum was recorded using the instrument Perkin-Elmer RXi spectrometer by KBr pellet method. The proton NMR and ^{13}C NMR spectrum of the compound were taken on the 300 MHz Bruker model spectrometer using $CDCl_3$ solvent and

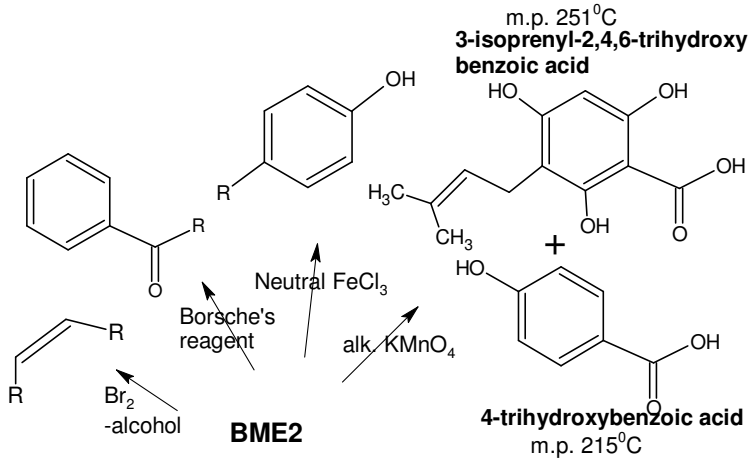
TMS standard. The GC-MASS spectral study of BME_2 was done using spectrometer JEOL GC Mate. The data are furnished in the Table-2.

TABLE-2
SPECTRAL DATA OF BME_2

Spectroscopy	Experimental data
UV-VIS spectroscopy (λ_{max}, nm)	216 (very intense), 260 (intense); 274 (less intense), 328
IR spectroscopy (ν_{max}, cm^{-1})	3542, 3040, 2924.58, 2854.9, 1633, 1566.8,1417, 1114, 661.9, 602, 472.5
^1H NMR spectroscopy (δ_{ppm})	1.53-1.67, 1.92-2.00, 4.21-4.25, 4.84-4.94, 5.29, 5.71, 5.79, 7.05, 7.29, 7.45, 7.64.
^{13}C NMR (δ_{ppm})	22.71, 31.44, 31.94, 38.84, 94.50, 105.41, 114.07, 120.24, 128.84, 130.92, 139.29, 154.89, 155.12, 155.29, 159.01, 169.02
Mass spectroscopy (m/z values)	342, 273, 255, 245, 152, 121, 110, 94, 69, 56, 55, 54, 52.

RESULTS AND DISCUSSION

The BME_2 isolated was purified and recrystallized out using ethanol. It was a pale yellow solid (m.p. 260-262 ºC and molecular mass 341.56). It was soluble in polar solvents like acetone, ethanol, ethyl acetate, acetonitrile, *etc*. By the tests with bromine reagent, Borsche's reagent and neutral ferric chloride reagent the presence of unsaturation, ketonic and phenolic groups were confirmed. The degradative oxidation using alkaline potassium permanganate reagent confirmed the phenoilc part was connected to the hydroxyl benzoyl part through a short carbon skeleton.



The UV-Vis spectrum showed the intense band at 216 nm signifies the $\pi \rightarrow \pi^*$ transition of an ethylenic double bond. The medium intense band at 274 nm indicated the $\pi \rightarrow \pi^*$ transition of the aromatic double bond. Another moderately in-

tense band at 260 nm was due to $\pi \rightarrow \pi^*$ transition in the carbonyl double bond. The $\pi \rightarrow \pi^*$ transition of the aromatic double bond of the phenolic part showed the band at 328 nm. Thus, the compound was found to have ethylenic double bond along with aromatic ring and the presence of the carbonyl double bond and the phenolic part[18].

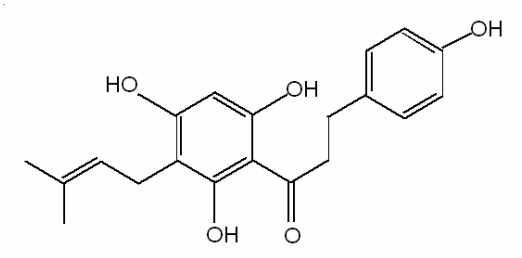
The IR spectrum[19] showed a strong band at 3452 cm^{-1} signifies the stretching of O–H bond that is involved in hydrogen bonding. The bending vibrations of the O–H bond was found through the bands at 1417 cm^{-1} and 602 cm^{-1}. The weak bands at 790 and 720 cm^{-1} were indicative of the bending vibrations of the C–H bond in ethylenic part and the C–H rocking vibration of the methylenic part, respectively. Another merged intense band at 3040 cm^{-1} indicated the C–H stretching of a aromatic ring and the C–H stretching of ethylenic part. The two bands at 2924 and 2854 cm^{-1} were indicative of the C–H stretching of methyl ($–CH_3$) and methylenic ($–CH_2–$) parts, two weak bands at 2372, 2116 cm^{-1} were due to –C=C– stretching. The C=O stretching of the carbonyl was shown at 1633 cm^{-1} as an intense band. The C=C stretching and C–H stretching were indicated by the bands at 1566 cm^{-1} and 114 cm^{-1}.

The proton NMR spectrum[20] gave a signal at 1.53-1.67 ppm (6H) indicating the presence of methyl protons. The methylenic proton ($–CH_2–$) gave a doublet signal at 1.92-2.00 ppm (2H) showed that it was slightly deshielded and had one neighbouring proton. The triplet signal at 4.21-4.25 ppm (1H) was indicative of a deshielded ethylenic proton –C=C– with two neighbouring proton. The singlet signal at 4.84-4.95 ppm (1H) signified the presence of deshielded and shielded proton present in the aromatic ring. The partially merged two triplet at 5.29 ppm (4H) signified the presence of two sets of deshielded methylenic protons that were present adjacently to each other ($–CH_2–CH_2–$). The two doublets at 5.71 and 5.79 ppm (2H, 2H) were due to the presence of aromatic protons that were partly shielded and deshielded. The four feeble signals at 7.05, 7.29, 7.45 and 7.64 ppm indicated the presence of four hydroxyl groups that were slightly deshielded.

The off-resonance and decoupled ^{13}C NMR spectrum[21] showed the signal at 31.3 ppm indicating the presence of methyl group ($–CH_3$) present in a mild deshielded environment. The –C=C– ethylenic carbons showed the signal at 139.24 and 120.24 ppm. The signal at 22.71 ppm indicated the presence of a methylenic carbon ($–CH_2$). The carbons that form the aromatic ring showed 6 signals in the range 94.50-159 ppm (94.50, 105.41, 154.2, 155.12, 159.01 pm). The highly deshielded carbonyl carbon showed signal at 169 ppm. The two methylenic carbon ($–CH_2–CH_2–$) that were in a slightly deshielded environment show the signal at 31.94 and 38.84 ppm. A set of signals in the range 114.07, 128.84 ppm were due to the two sets of aromatic carbons to each other one *meta* and the other set *ortho* to the carbon with –OH group, respectively. The aromatic carbon *para* to the carbon with –OH group showed a signal at 130.92. The aromatic carbon bearing –OH function in highly deshielded and showed a signal at 155.21 ppm.

The mass spectrum[22] gave the M^+ value at 342 indicating the presence of molecular ion. The base peak at 51.5 showed the existence of the stable cyclobutadiene species. An intense peak at 69.1 was due to the presence of an isoprenyl part. The other signals at 273, 245, 152, 121, 110, 94, 93 56, 54 were indicative of the presence of varies daughter fragments. The existence of those daughter fragments were shown in the mass spectral fragmentation pattern. The m/z values of these daughter ions were in agreement with the m/z values observed in the mass spectrum.

Based on the chemical and spectroscopic studies the structure of BME_2 is deciphered to be a chalcone as shown below:



5-Isopreny-2,4,6-trihydroxy-α-(*p*-hydroxybenzyl) acetophenone

The structure may be confirmed more authentically by X-ray diffraction study.

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BIRMINGHAM, UNITED KINGDOM, EUROPE

Contact:
Third Floor, Eagle House, 16 Proctor Street, London WC1V 6NX, UK
E-mail: conferences@biochemistry.org; elizabeth.faircliffe@biochemistry.org
Tel: +44 (0) 20 7280 4150 Fax: +44 (0) 20 72804167
Web Site, http://www.biochemistry.org/MeetingNo/SA106/view/Conference/