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A Phytochemical Analysis of the Medicinal Plant: *Anisochillus carnosus*

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The aerial parts of the plant, *Anisochillus carnosus* were analyzed phytochemically and a compound was isolated from the ethyl acetate extract. The compound was characterized, employing chemical and spectral methods, to be a 3,5,7,4′-tetrahydroxy-8-isoprenyl flavonoid.

Key words: Phytochemical, analysis, *Anisochillus carnosus*.

INTRODUCTION

The phytochemical work on this medicinally useful plant is much less available. The juice of the plant's leaves is used to relieve liver disorders and other allergic symptoms. The leaves are used as food in Bihar region of India. The reddish brown oil of this plant is found to have muscle relaxant action and also bactericidal and fungicidal properties. The plant is used as a cure for cold and fever in Indian folk medicine[1–4]. The medicinal properties may be due to the active metabolites that are present in this plant. The flavonoids like luteolin and apigenin were isolated from this plant. With a view to isolate and identify some active compounds, the present work was taken up.

The compound was isolated by chromatographic separation technique from the ethyl acetate extract. Later, it was analyzed chemically and spectroscopically to identify the flavonoid compound.

EXPERIMENTAL

Plant Material: The aerial parts of the plant were collected from the Palni Hills, Tamilnadu, India during June and July. The plant specimen was verified[5, 6] with the Rapinat Herbarium, St. Joseph's College (Autonomous), Tiruchirapalli, India.

Extraction: The plant materials were cut into pieces, shade-dried and soaked with ethanol-water solvent mixture (EtOH : H_2O, 4 : 1) for 48–62 h. The extract was filtered, concentrated and treated with a small amount of 2 M hydrochloric acid. The acidic extract was further extracted with solvents like chloroform, ethyl acetate, etc. one by one in the increasing polarity order. The ethyl acetate extract thus obtained was chromatographed by TLC technique using a three solvent mixture as eluant and silica gel (100 micron) as stationary phase. Several compounds were found to separate out. One of these compounds was isolated and taken for the present study. It was a pale yellow solid about 900 mg in yield, and was labelled ACE1.

General: The compound ACE1 was recrystallized from alcohol. Then, it was

chromatographed[8] on Whatmann-3 filter paper using the eluant, butanol-acetic acid-water mixture (4 : 1 : 5) to get a single spot. The R_f was found to be 0.81.

The solubility of the compound was tested in different solvents like acetone, dimethyl sulfoxide, ethanol, ethyl acetate, etc. Also, the compound was soluble in hot water and alkali. The melting point of the substance was found to be 239–241°C using the melting point apparatus (model: Gallemkamp, British made)[9].

About 10 mg of the substance was dissolved in ethanol and on treatment with 5 mL 5% ferric chloride it gives pink coloration.

About 20 mg of the substance was dissolved in 2 mL chloroform. This solution was treated with 2 mL bromine-$CHCl_3$ reagent. An orange coloured precipitate was formed.

20 mg of the substance was dissolved in 1 mL ethanol and mixed with 2 mL phenylhydrazine reagent and warmed gently. Yellow precipitate of phenyl hydrazone was formed.

To 20 mg of the compound ACE1, about 1 mL acetic anhydride was added. The contents were mixed with 5 mL pyridine. Then it was treated with 50 mg sodium acetate and a small quantity of acetyl chloride. Heated on a steam bath for 3–4 h. The colourless solid formed was recrystallised from ethanol-acetone solvent mixture. It was found to have the melting point 234–236°C.

About 20 mg of the substance was dissolved in 15 mL acetone and 50 mg potassium carbonate and 10 mg methyl sulfate were added. Heated the reaction mixture for 3–4 h in a steam bath. The mixture was filtered. The filtrate was evaporated to dryness. The colourless solid formed was recrystallized from ethanol. Its melting point range was found to be 141–143°C. This product responded positively showing green coloration with ferric chloride reagent. Thus, the compound was partially methylated.

Nearly 20 mg of the substance was mixed with 20 mg methyl sulfate and 50 mg potassium carbonate. The whole contents were put into 30 mL acetone. Heated on a steam bath for 48 h. The mixture was filtered. The filtrate was evaporated to dryness. The colourless solid formed was recrystallized from ethanol. Its melting point range was found to be 109–112°C. This product did not respond positively showing any colour with ferric chloride reagent. Thus, conclusively, the compound was totally methylated[10].

About 20 mg of the substance ACE1 was treated with 2 mL 20% alcoholic potassium hydroxide in nitrogen atmosphere and heated for 4 h. The excess solvent was distilled off under reduced pressure. The residue was dissolved in water and saturated with carbon dioxide[11] and then extracted with ether. The ethereal solution was treated with sodium bicarbonate solution. Two layers were formed. The aqueous layer was separated and neutralized with 2 N HCl. The colourless solid that was precipitated was found to be an acid. It was recrystallized by hot water filtration. The melting point was found to be 213–215°C. It was mixed with *p*-hydroxy benzoic acid and the melting point was measured. There was no dip in the melting point. A proton-NMR spectrum was also taken for this compound.

The ethereal layer was evaporated gradually to get an orange solid. It was recrystallized from ethanol. The melting point was 201–203°C. It was also taken for proton-NMR spectral study. This compound responded positively for the

ketonic functionality with the reagent, semicarbazide. It was identified to be a polyhydroxy acetophenone type of compound.

About 20 mg of the totally methylated ACE1 was subjected to hydrolysis by the same method as described above; the products formed were *p*-methoxy benzoic acid (m.p. 182–184°C) and hydroxy, methoxy acetophenone type of compound (m.p. 98–101°C). The proton-NMR spectrum was also taken for this compound.

A known amount of the substance ACE1 (20 mg) was dissolved in 50 mL of ethanol . The solution was taken in the cell or sample tube of the polarimeter (Model: Drossel, Carl Zeiss). The transmitted light was analysed through the analyser. The compound was observed to be optically inactive.

The compound ACE1 was subjected to C-H-N analysis using the instrument Heraeus CHN rapid analyzer. Based on the report the empirical formula was calculated to be $C_{3.33}H_{2.99}O_1$. The molecular formula was $C_{20}H_{18}O_6$. The percentage composition of the elements is given in Table-1. The molecular mass of the compound was determined by cryoscopic method[9]. It was calculated to be 354.1152.

The UV-Vis, IR, proton-NMR and mass spectral studies were done for this compound ACE1 at RSIC, IIT, Chennai. The ^{13}C-NMR study was carried out at SIF, IIS, Bangalore. The UV-Vis spectrum was taken using the instrument Varian Cary 5E model. Spectroscopic grade ethanol was used. The IR spectrum was recorded on Bruker IF 66V FT-IR spectrophotometer by the pellet with KBr. The instrument Jeol GSX 400NB was used to record the proton-NMR spectrum. DMSO-d_6 solvent was used to prepare the solution. Tetramethyl silane (TMS) was the standard. The ^{13}C-NMR spectrum was recorded using the spectrometer of 100 MHz Brucker model. The mass spectral study was done with the spectrometer Finnigan 8230 MS.

The spectral data of the various spectral studies are furnished in Table-1.

TABLE-1
EXPERIMENTAL DATA OF ACE1

Type of Experiment	Data
Cryoscopic method	Molecular mass 354.1152
C and H analysis	(%)C 67.7694; H 5.0887
UV-Vis spectroscopy,	
λ_{max}(nm)	210, 266, 294sh, 322sh, 367
$\varepsilon_{max}(10^3\ cm^2\ mol^{-1})$	23730, 16740, 9580, 5360, 14360
IR spectroscopy, ν (cm^{-1})	3360, 3335, 3070, 3055, 3015, 3000, 2958, 2865, 1705, 1685, 1670, 1650, 1610, 1580, 1515, 1480, 1468, 1460, 1390, 1370, 1330, 1250, 1040, 890, 748, 720, 690, 650
^{1}H-NMR spectroscopy, δ(ppm)	1.78, 2.3d (J 10), 5.77, 5.84t (J 9), 6.85d (J 9), 7.76d (J 8), 7.82d (J 6), 9.36, 9.68, 11.12, 12.68
^{13}C-NMR spectroscopy, δ(ppm)	20.8, 24.1, 95.3, 102.9, 108.2, 116.2, 118.1, 120.6, 133.7, 135.5, 137.3, 144.6, 156.2, 160.1, 161.5, 167.7, 175.3
Mass spectrometry, M/z	354, 286, 261, 233, 221, 193, 190, 177, 176, 152, 136, 122, 121, 120, 94, 93, 70, 68, 58, 42, 40, 30, 26

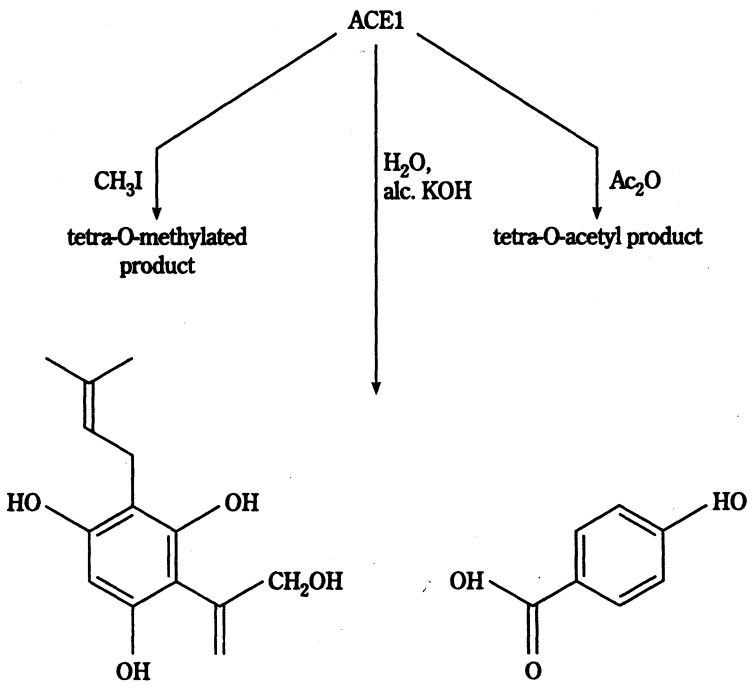
RESULTS AND DISCUSSION

The yellow solid (m.p. 239°C, mol. mass. 354) gave positive test for the flavonoid.

The compound reacted with phenyl hydrazine reagent to give an intense yellow precipitate of phenyl hydrazone. This confirmed the presence of ketonic group. The bromination test gave an orange-coloured precipitate indicating the presence of unsaturation. With the methyl iodide reagent it showed tetra-methylated product (m.p. 109–112°C). Acetylation of the compound gave a tetraacetylated product (m.p. 233–235°C). Thus, the presence of four hydroxyl groups were confirmed.

Hydrolysis of ACE1 gave two products: P1 and P2. P1 was identified to be a *p*-hydroxybenzoic acid. It was confirmed by mixed-melting, and co-TLC with the authentic sample. The product P2 was found to be hydroxy acetophenone type of compound (m.p. 204–207°C) . The proton-NMR spectrum of this compound suggested that it was trihydroxy and ω-hydroxy acetophenone with an isoprenyl substituent.

Scheme-1, illustrates the aforesaid chemical transformations of ACE1.



Scheme-1. Chemical transformation of ACE1

The UV-Vis spectrum (Fig. 1a) of ACE1 showed at λ_{max} 294 sh, 322 sh and 367 nm, band-I and 210, 266 nm, band-II characteristic of a flavonoid[12, 13]. With the shift reagent NaOMe, the band-I shifted to 318, 332 and 414 nm and without

any marked increase in the intensities. This signified the presence of hydroxyl groups at 3 and 4′ positions of a flavonoid.

The shift reagent NaOAc brought about the shift of band-II to 228 and 286 nm. This signified the presence of the hydroxyl group at the 7 position.

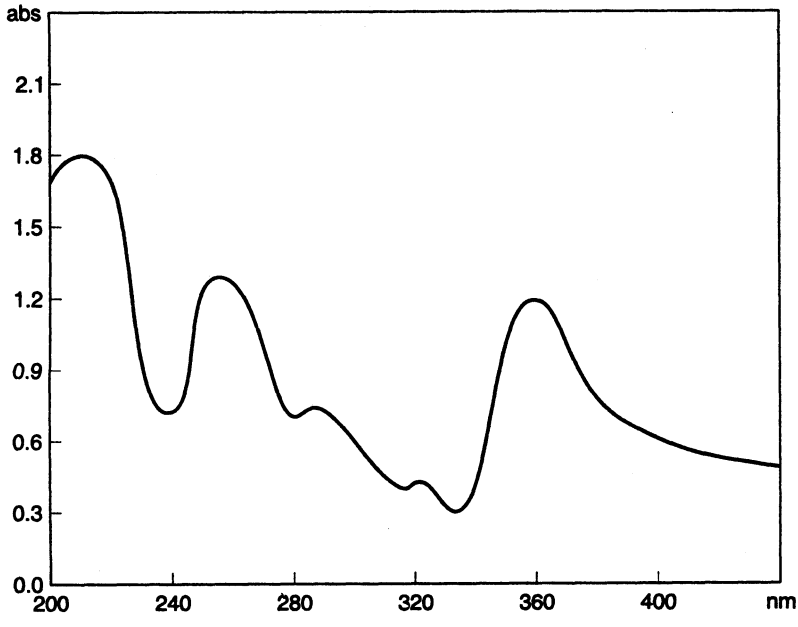


Fig. 1a. UV-Vis spectrum of ACE1

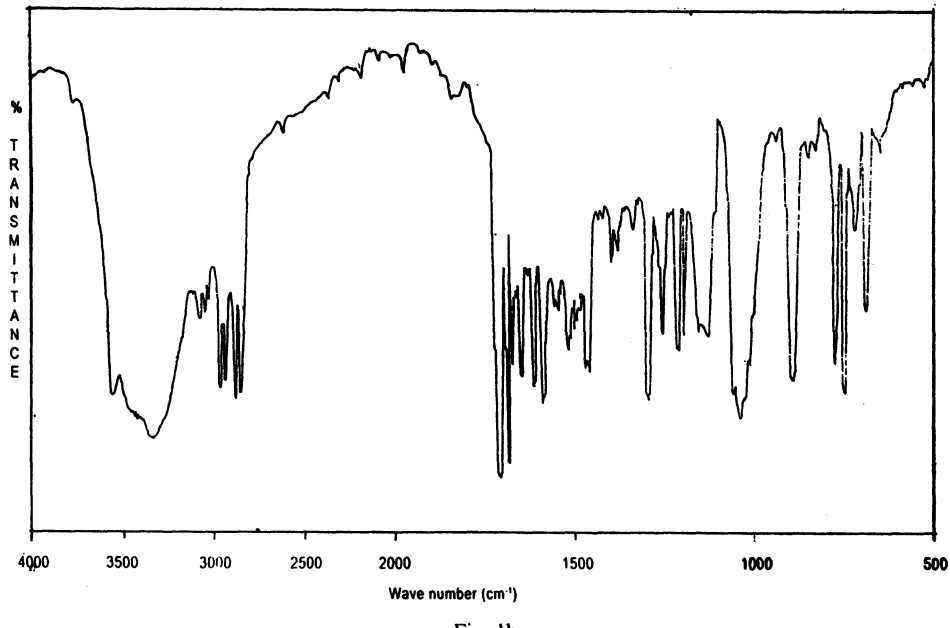


Fig. 1b

The use of $AlCl_3$ and HCl effected a bathochromic shift of band-I to 421 nm, indicating the presence of 5-OH in the flavonoid.

In the IR spectrum[14] (Fig. 1b) a very intense band at 3560 cm^{-1}, intensely broad band at 3335 cm^{-1} and moderately intense bands at 1390 and 650 cm^{-1} were observed for the O—H bond vibrations of hydroxyl group. The out-of-plane and in-plane C—H vibrations of the unsaturated part were observed as strong bands at 3070 and 890 cm^{-1}. The corresponding C=C vibrations were shown around 1489 and 1670 cm^{-1} as weakly intense bands. The C—H bond vibrations of the aromatic part occurred in the region 3055, 3015 and 3000 $cm^{-1}$and 740 cm^{-1}. The stretching and bending vibrations of methyl part were noticed by the intense bands 2958 and 2865 cm^{-1} and medium intensity bands at 1460 and 1370 cm^{-1}. The vibrations of the methylenic part were shown by the bands at 2930 and 2845 cm^{-1} and the medium band at 1468 cm^{-1}. The moderately intense band at 720 cm^{-1} was attributed to the rocking movement of methylenic part. The carbonyl group showed its C—O stretching vibration at 1705 cm^{-1}. The C=H bond stretch of the aromatic ring occurred around 1685, 1650, 1610, 1580 and 1515 cm^{-1}. The bending vibrations of the same C=C bond were identified with the moderately intense bands around 690 cm^{-1}. The stretching movement of the C—O—C bond was shown by the medium bands around 1250 and 1040 cm^{-1}.

The proton-NMR spectrum[15] (Fig. 1c) showed that the two quartet signals at 1.78 ppm (J 4 Hz) in the merged form might be due to the presence of two methyl groups attached to the same carbon of an unsaturated part. The doublet at 2.3 ppm (J 10 Hz) was shown by a methylenic proton. The triplet signal with J 9 Hz and a singlet signal with J 3 Hz had merged around 5.7 ppm. These were due to

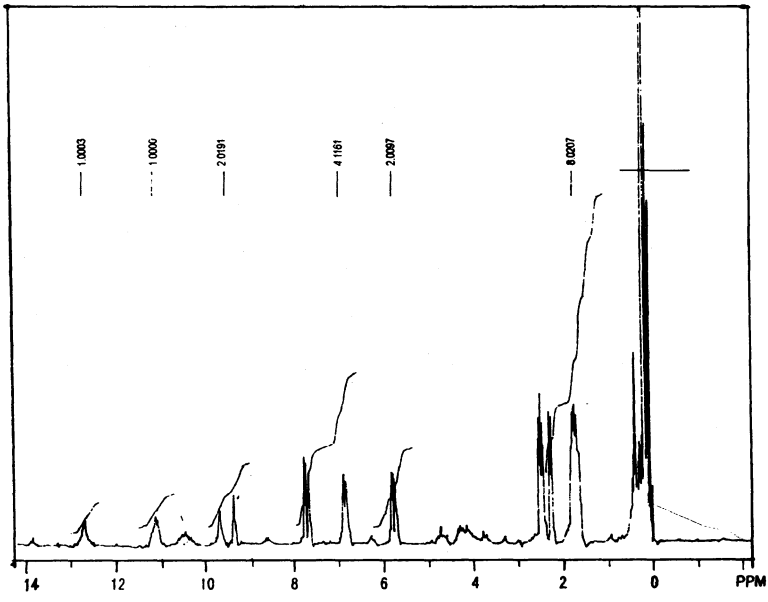


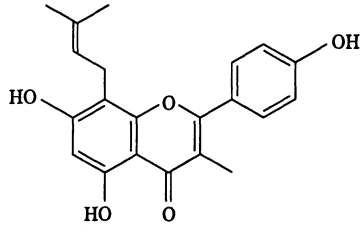
Fig. 1c. Proton-NMR spectrum of ACE1

the methylenic and aromatic protons, respectively. The doublet peak at 6.85 ppm (J 9.6 Hz), was shown by the two aromatic protons, which had direct coupling with one adjacent proton each. Another doublet signal at 7.7 ppm with the coupling constant J 7.2 Hz might be due to the two other aromatic protons, which were coupled with two adjacent protons each. Four hydroxyl protons gave the four singlet signals at 9.36, 9.68, 11.1 and 12.68 ppm. Two of these OH protons were supposed to be in high deshielding and far downfield.

A fully decoupled ^{13}C NMR spectrum[16, 17] of ACE1 gave information about the carbon skeleton. The signal at 20.8 ppm was for the methyl carbon. The methylenic carbon showed its signal at 24.1 ppm. The six carbon atoms of aromatic ring showed signals at 95.3, 102.9, 108.2, 160.1, 161.5 and 167.7 ppm. Three of these six carbons showed high δ values, as they were very much deshielded. These carbons were said to experience the electron-withdrawing influence by OH groups. Two alkenic carbons showed signals at 144.6 and 137.3 ppm, of which one with a high δ value was understood to be highly deshielded. Another set of alkenic carbons gave signals at 120.6 and 131.7 ppm. The four signals at 118.1, 116.2, 133.7 and 156.2 ppm signified the presence of four kinds of carbons in the aromatic ring. One of these carbons was highly deshielded with high δ value and the other two were moderately deshielded, might be experiencing electron-withdrawing influence by —OH groups. The signal with 175.3 ppm value was felt due to the carbonyl carbon.

From these experimental findings the compound ACE1 was identified to be 3,5,7,4′-tetrahydroxy-8-isoprenyl flavonoid.

The proposed structure was further confirmed by the mass spectral study[15, 16].



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