

A New Furocoumarin from the Leaves of *Camellia sinensis*(L.) O. Kuntze

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Abstract – From the methanolic extract of defatted tender leaf of *Camellia sinensis* a new 4-hydroxy angular furocoumarin $C_{12}H_8O_5$, m.p. 212 °C, was isolated using high-speed counter-current chromatographic technique. The structure of the compound was established as 4-hydroxy-2'-methoxy angular furocoumarin on the basis of physical methods *viz.* 1H NMR, ^{13}C NMR and MS.

Key words – *Camellia sinensis*(L.) O. Kuntze, Theaceae, methanolic extract of the leaf, 4-hydroxy-2'-methoxy angular furocoumarin.

Introduction

In continuation of our work on the isolation and characterization of active chemical constituents in tender green leaves of *Camellia sinensis* (Ray and Ganguly, 1988) we wish to report the isolation and characterization of a new angular furocoumarin from the methanolic extract of the defatted tender leaves of *C. sinensis*.

Experimental

General experimental procedures – Melting point is uncorrected. High-speed counter-current chromatograph operates at 2000 rpm under 80-100 psi (Pharma Tech. Research Corp., U.S.A., Model CCC-3000). IR spectrum was obtained with Hitachi 260-10 Infrared Spectrophotometer, UV spectrum of the compound was studied with spectral ethanol using Beckmann DK 2 Ultraviolet spectrophotometer. 1H and ^{13}C NMR spectra were measured with TMS as internal standard employing Bruker AF-200 NMR spectrophotometer operating at 200MHz using $CDCl_3$

as solvent.

Plant material – The leaves of *Camellia sinensis* were collected from Mornai Tea Estate, Assam, India, in August 1992.

Extraction and Isolation – The air dried leaves of *C. sinensis* (1 kg) was defatted with petroleum ether (60-80 °C). The defatted leaves were further extracted with methanol. The methanolic extract on concentration yielded a dark green tarry mass. It was taken up with ether and the solution was extracted with aqueous alkali (3%). The alkali extract on acidification with hydrochloric acid in ice cold condition yielded a light green precipitate which was further extracted with ether. The ethereal extract was then washed with water till free from acid and dried over anhydrous sodium sulphate. It was then filtered from sodium sulphate. The filtrate was evaporated and the residue left was taken up with chloroform. The chloroform solution was subjected to thin layer chromatography (TLC). Next, the spot of interest was taken up for thin layer chromatography-chemical ionization mass spectroscopy (TLC-CIMS) (Tabei, 1991). The TLC-CIMS is an unique method in which the sample is directly taken from the TLC plate with the help of a

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glass needle previously coated with carbowax of molecular weight 20 million (approx). Then the glass needle with the sample sticking on the tip is put directly into the mass spectrum inlet chamber. The reagent gas used was methane. Finally, for isolation of the compound, the technique of high-speed counter-current chromatography (HSCCC) (Zhang *et al.*, 1988) was employed, which is one of the latest in high resolution chromatographic techniques which utilizes a free liquid partitioning method taking advantage of the strong centrifugal force fields to isolate compounds present in the plant extract even in microgram scale.

Results and Discussion

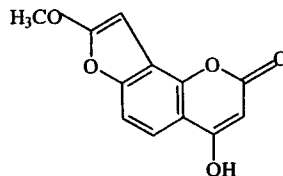
The acidic part of the methanolic extract of the leaf of *C. sinensis* was subjected to TLC using petroleum ether: benzene (2:1) as developing solvent on silica gel G plate. On spraying the plate with alcoholic alkali, a yellow spot was observed at Rf. 0.39, indicating the presence of a coumarin derivative in the extract besides other compounds.

The spot corresponding to Rf. 0.39 was further examined before spraying by TLC-CIMS. The TLC-CIMS showed the molecular weight of the compound to be 232 (M^+).

To isolate the above compound the entire methanolic extract was subjected to HSCCC. The solvent system used for isolation was ethyl acetate/chloroform/methanol/water (1:3:3:2). The coumarin was isolated as a white solid (yield 0.0001%) which was crystallized from chloroform-petroleum ether mixture, m. p. 212 °C. It was found homogenous in TLC.

The high resolution mass spectrum showed the molecular formula of the compound to be $C_{12}H_8O_5$ (M^+ 232). The other important peaks are at m/z 217, 202, 190 and 185. The UV-spectrum of the compound in ethanol showed absorption maxima at 252 (logT 4.31) and 302 (logT 3.84) characteristic for angular furocoumarin. The IR spectrum in nujol

showed bands at 3490 (-OH group), 1718(α , β -unsaturated δ -lactone), 1620(-OCH₃ group), 1660, 1452, 1375, 1345 (aromatic residue) and 890 cm^{-1} (furan ring). The ¹H NMR spectrum in CDCl₃ showed a singlet at δ 7.03 for H-3' proton, The H-5 and H-6 appeared as doublet at δ 7.34 and δ 7.44 respectively and a singlet appeared at δ 4.27 for three protons indicating the presence of one methoxy group. The position of the methoxy group was fixed at C-2' in the furan ring of the coumarin nucleus on the basis that the H-3' appeared at much higher field. The absence of a pair of doublet for H-3 and H-4 protons clearly indicates the presence of substitution either at 3- or 4- or both in the coumarin nucleus (Steck and Mazurek, 1972), The appearance of a singlet at δ 5.98 for one proton clearly indicates the presence of H-3. The hydroxy signal appeared at δ 3.62. Thus, the hydroxy group and methoxy group were placed in 4 and 2' position respectively. The ¹³C NMR of the compound showed clearly the presence of 12 carbon atoms, with important peaks at δ 51.93 (-OCH₃), 154.6 (C-2), 112.6 (C-3), 109.6 (C-2'), 144.23 (C-3'), 165.3 (C-7), 107.5 (C-8), 122.1 (C-6) and 112.25 (C-5) (Levy *et al.*, 1980). On the basis of the above findings the structure of the compound was given as,



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