



Phytochemical studies on the isolation of 6-hydroxy-7,2',4'-trimethoxy isoflavone

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ABSTRACT

From the ethylacetate: methanol fraction of the cassia occidentalis, 6-Hydroxy-7,2',4'-trimethoxy isoflavone, was isolated. The isolated compound was characterized by UV, IR, ¹H NMR, ¹³C NMR and mass studies.

1. Introduction

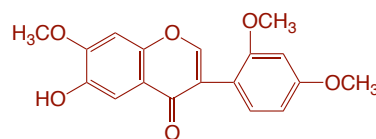
Compound-1, florescent, greenish yellow in colour m.p. 210°C was analysed for C₁₈H₁₆O₆ [M⁺:328]. The homogeneity and purity of the compound was ascertained by TLC. The compound gave the following colour reactions characteristic of isoflavones. It gave green colour with ferric chloride solution showing its polyphenolic nature¹. It gave pink colour with sodium amalgam and hydrochloric acid². It reacted slowly with magnesium and hydrochloric acid to give yellow solution³. It gave yellow colour with conc. sulphuric acid⁴, with a characteristic fluorescence. These colour reactions confirmed the isoflavonoid nature of the compound. The possible nature of the compound as an isoflavone was further confirmed by its UV spectrum, showing an absorption maximum of high intensity at 260 nm (Band II) with only an inflection of low intensity at 280 nm (Band I). This is the characteristic of isoflavone which absorb in the region of 245-270nm⁵.

IR spectrum showed an absorption peak of hydroxy group at 3450 cm⁻¹ and two absorption bands at 1650 and 1590 cm⁻¹ for an α β -unsaturated Ketone. IR spectrum showed an absorption peak of methoxy group at 2880 and 1185 cm⁻¹. The compound on acetylation with acetic anhydride and fused sodium acetate formed a mono acetate there by confirming the presence of only one hydroxyl group in the compound. ¹H NMR spectrum of the compound showed the signals of δ 3.68 (3H, s), 3.78 (3H, s) and 3.92 (3H, s) corresponding to three methoxyl groups. This was also supported by ¹³C NMR spectrum which showed the signals at δ 57.00, 57.82 and 58.02 respectively. On methylation, compound gave a methyl ether which analyzed for four methoxyl groups. Since three methoxyl groups were already present in the compound, one phenolic hydroxyl group present in the compound must have got methylated.

Compound contained six oxygen atoms; these were accounted for as-one in the hydroxyl form and two in the isoflavone

nucleus. The remaining three must be present in the form of three methoxyl groups. The relative positions of hydroxyl and methoxyl groups were further confirmed on the basis of colour tests and spectral studies. The compound gave the negative test with Boric acid in presence of critic acid⁶ and Zirconium oxychloride⁷ indicating the absence of a free hydroxyl group at position-5 of ring A. The compound did not impart pink colour with vanillin-hydroxyl acid reagent⁸ indicating the absence of 1, 3-dihydroxyl system.

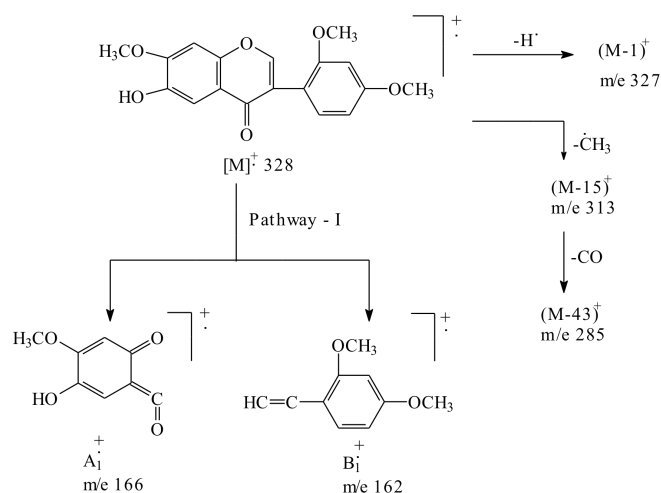
¹H NMR spectrum of the compound showed the presence of six protons in the aromatic region. ¹H NMR spectrum exhibited a signal at δ 7.56 (1H, s) as singlet which was assigned for C-2 position⁹. This was also supported by the ¹³C NMR spectrum¹⁰ which showed a signal at δ 154.20 (d). The doublet at δ 7.38 (1H, d) and double doublet δ 6.38 (2H, dd) were assignable to C-6', C-3' and C-5' respectively. The multiples of these signals indicated that the C-3' and C-5' protons were meta coupled. ¹H NMR spectrum of the compound also showed a sharp singlets at δ 6.74 (1H, s) and 6.51 (1H, s) which were assigned for C-5 and C-8 protons of ring-A respectively. The singlet at δ 12.32 (1H, s, OH) was assignable to the proton of hydroxyl group at C-6. Thus, the positions left for the substitution of three methoxyl groups were C-2', C-4' and C-7 respectively. On the basis of above spectral data, the final structure of the compound can be identified as: - 6-Hydroxy-7, 2', 4'-trimethoxyisoflavone.



Compound 1

Further, the above structure was finally confirmed by mass fragmentation pattern, which was the characteristic of isoflavone and is represented as follows:

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2. Plant Material

Cassia occidentalis (Syn. *Senna occidentalis*) belongs to Family Leguminosae and Subfamily Caesalpinieae commonly known as Badikasondi, Chakunda, Kasonda in Hind.¹⁰ The plant is generally distributed throughout India and the tropics.¹¹ The whole plant of *Cassia occidentalis* was collected from Allahabad, U.P India. The specimen was identified by Prof. D.K. Chauhan Department of Botany, University of Allahabad, India-211002.

3. General Procedures

All the spectral studies throughout work have been done using the instruments as follows. UV Spectra were recorded on a Beckmans-DK2 Spectrophotometer. IR spectra were recorded in KBr on a Perkin Elmer spectrometer. 1H NMR spectra of compounds CO-1 was recorded at 300 MHz and ^{13}C NMR at 100 MHz in $CDCl_3$. Mass spectra were recorded on a JEOLJMS-D300 mass spectrometer. Column chromatography¹² was carried out using silica gel (60–120 mesh). Chemicals of analytical-reagent grade were purchased from E-Merck (India).

Extraction and Isolation

The air dried and finely crushed whole plant of *Cassia occidentalis* was defatted by extraction with boiling hexane under reflux. The hexane defatted plant was subsequently extracted exhaustively with ethanol under reflux. The alcoholic extract was concentrated under reduced pressure in a rotatory evaporator and poured into a ice-cold distilled water with constant stirring, a dark brown aqueous solution (Fraction-I) and a light brown residue (Fraction-II) were obtained which were separated by filtration.

Fraction-I was concentrated under reduced pressure. The concentrate was loaded over a flash column and eluted with hexane, benzene, ethyl acetate and methanol respectively in the order of their increasing polarity. From the ethyl acetate: Methanol (9:1, v/v) one compound was obtained.

References and notes

1. M. Shimimi, *J. Pharm. Soc.*, Japan, **1951**, 71, 1339.
2. V.S.S. Murti, S. Rogopalan and L.R. Row, *Proc Indian Acad. Sci.*, **1951**, 34, 319.
3. J. Shinoda, *J. Pharm Soc. Japan*, **1928**, 48, 412.
4. C. Graebe, *Annalen*, **1906**, 211, 349.
5. R.H. Thomson, 'Naturally Occurring Quinones', (Academic Press, London & New York) 2nd ed. **1971**, 55.
6. F. Feigl and V. Anger, 'Spot Tests in Organic Analysis', 7th English edit. Elsevier, **1966**.
7. J. Lemli, R. Sequeker and J. Cuveele, *J. Pham Weekblad*, **1964**, 99(14), 351.
8. H. Raistrich, R. Robinson and A.R. Todd, *Biochem. J.*, **1934**, 28, 559.
9. J.C. Pew, *J. Am. Chem. Soc.*, **1948**, 70, 3031.
10. H. Raistrich, R. Robinson and A.R. Todd, *Biochem. J.*, **1934**, 28, 559.
11. T.A. Geissman, *T.A. in the chem. of Flavonoid Compounds, INC. New York.*, **1962**, 72.
12. T.A. Geissman, Modern Methods of Plant Analysis, edited by K. Peach, M.V. Tracey, *Jalius Springer, Berlin*, **1955**, 3, 450.