

Novel Potential Antiviral; - 5-Hydroxy 3, 7, 8, 5' Tetramethoxy 3', 4' – Methylenedioxy Flavones from the Fruits of Citrus Reticulate Blanco

Dr. Preeti Khare, Dr. Narender Kumar
Corporate Institute of Science & Technology
Bhopal (M.P.)

Dr Tarun Kumari
Trinity Institute of Science & Technology
Bhopal (M.P.)

ABSTRACT:

A novel flavones, 5- hydroxy 3, 7, 8, 5' – tetramethoxy 3', 4' – methylenedioxy flavones 1 has been isolated and identified by chemical degradations and spectroscopic methods.

INTRODUCTION:

Citrus species (N.O. Rutaceae) are cultivated in all subtropical regions of the world (1). The CHCl_3 soluble fraction of the methanolic extract of the fruits of citrus reticulate on column chromatography over Si-gel afforded three flavonoidal compounds in successive eluants C_6H_6 : CHCl_3 (4:1, 2:3, 1:1). The structure of compound has been established as; 5-hydroxy 3,7,8,5' – tetramethoxy 3', 4' – methylenedioxy flavones on the basis of uv, ir, ^1H -nmr, ^{13}C -nmr, mass and alkaline degradation.

RESULTS AND DISCUSSION:

The dried and powdered fruits of citrus reticulata Blanco were exhaustively extracted with methanol. The concentrated methanolic extract gave brown gummy mass under reduced pressure. The brown gummy mass on column chromatography over sig-gel afforded yellow crystals of compound with benzene: Chloroform (2:3) eluants. C_6H_6 (1:1) eluants gave compounds 2 and 3. The compound 1 $M^+ = 402$ ($R_f = 0.63$, Toluene: Ethyl Acetate

Formic Acid 5:4:1) Molecular formula $\text{C}_{20}\text{H}_{18}\text{O}_9$, m.p. – $187=189^\circ$ gave all positive colour reactions for flavonoid. The uv spectrum in methanol showed strong absorption bands at 264 and 363 nm. Presence of C-5 hydroxy group indicated by the fact that Aluminum chloride induced bathochromic shift of 31 nm in Band I (2) which gave a bathochromic shift of 55 nm in Band I on addition of HCl suggesting the presence of –OH group at C-5 and a methoxy group at C-8 (3).

The ir spectrum of the compound showed the absorption bands at 3335 cm^{-1} (hydroxyl group), 1676 cm^{-1} (conjugated carbonyl system), 930 cm^{-1} (methylenedioxy group) and 2870 cm^{-1} (methoxy group). Estimation of methoxy group (30.75%) by Zeisel's method (4) indicated the presence of four methoxy groups in compound 1. The ^1H -nmr spectrum (270 MHz CDCl_3) showed three singlets for four methoxy groups at 3.85 (s, OMe, 5'-3H) 3.88 (s, 2 OMe-7,8 6H) and 4.0 (s, OMe,-3H, 3H). A sharp singlet of proton intensity at 6.42 was assigned for C-6 proton. Two doublets each of one proton intensity were observed at 7.81 and 7.88 ($J = 1.5\text{ Hz}$) in ^1H -nmr indicated the presence of 2, -H and 6' –H protons of 3',4', 5' – trisubstituted B-ring. Another signal at 5.98 (2H) of two proton intensity was observed due to 3', 4' – methylenedioxy group at C-3' and C-4' of ring B. The ^{13}C nmr spectrum of compound was in accord with the proposed formula. The ^{13}C -nmr spectrum (CDCl_3) is given in Experimental.

The relative positions of methoxyl and methylenedioxy groups as shown in 1 followed from the mass spectrum which showed RDA fragments at m/e 181 (40%) and 206 (34%) were in confirmatory with the structure .

The structure of compound was finally confirmed by its alkaline hydrolysis, which yielded two neutral products. The more polar one was identified as 3-methoxy 4,5-methylenedioxy acid $C_9H_8O_5$, m.p. 208° , $M^+ = 196$ which is a well known compound, 3,4-dimethoxy 1 methoxy acetophenone $M^+ 242 C_{11}H_{14}O_6$ identified by Co-pc and Co-tlc.

EXPERIMENTAL:

GENERAL EXPERIMENTAL METHODS- Mps. Uncorr. NMR spectra were measured using TMS as an int. standard and $CDCl_3$ as solvent. IR spectra were measured in KBr-discs.

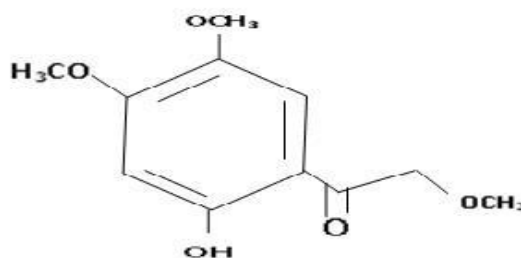
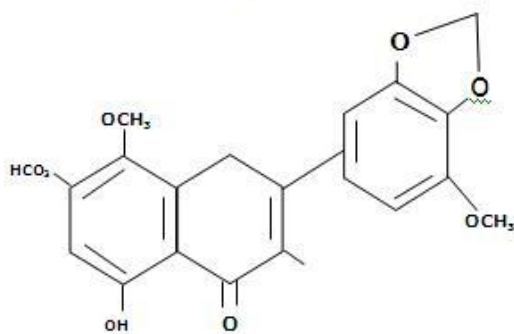
EXTRACTION AND ISOLATION: The air dried and powdered fruits (2.5 kg) of citrus reticulate were homogenized and extracted with 95% MeOH concentrated under reduced pressure. The concentrated extract was poured into excess of distilled water. The water soluble part was concentrated to a brown gummy mass. It was successively extracted with Petroleum ether, C_6H_6 , $CHCl_3$ EtOAc and MeOH.

The $CHCl_3$ fraction (8.2 g) was subjected to Si-gel chromatography developed successively with $C_6H_6 : CHCl_3$ in various proportions (4:1, 2:3, 1:1) The fractions collected from 2:3 portions gave on crystallization a light yellow compound (2.40 gm) which gave a single spot on TLC examination over Si-gel using Toluene : Ethyl acetate : Formic acid (5:4:1)

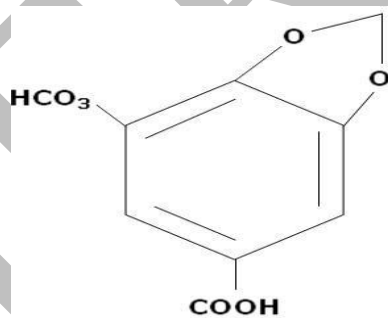
5-HYDROXY3,7,8,5'-TETRAMETHOXY3',4' METHYLENEDIOXYFLAVONE 1:

Yellow needle shaped; m.p. $187-189^{\circ}$, ir max (KBr) 3335, 2896, 1676, 1601, 1285, 1150, 930 (O-CH-O), 830, 2870 cm^{-1} uv / max nm 264 sgm 363 (MeOH); 262, 394 ($AlCl_3$); 287, 268, 262 418 ($AlCl_3/HCl$); 266, 368 (NaOAc); 277, 363 (NaOMe); 266, 363 (NaOAc/ H_3BO_3). 1H -nmr (270 MHz, $CDCl_3$) 6.42 (1H, s, H-6); 3.85, 3.88, 4.0 (3H, 6H, 3H, s, 4OMe); 5.98 (2H, s-O-CH₂-O-); 7.81 (1H, d, J = 1.5 Hz, H-2'), 7.88 (1H, d, J = 1.5 Hz, H-6'); EIMS data m/e : 402 (M^+ 15%) (Calc. for $C_{20}H_{18}O_9$ 402); 401 (M-H, 14-2%), 387 (M-Me, 22%) 374 (M-CO, 28%) 179 (32%) (Found C, 59.61; H, 4.37 calcd C, 59.70; H, 4.47%) ^{13}C -nmr (400 MHz, $CDCl_3$) 161.48 (C-2), 131.52 (C-3), 174.14 (C-4), 153.50 (C-5), 137.36 (C-6), 146.51 (C-7), 137.28 (C-8), 145.21 (C-9), 112.86 (C-10), 123.60 (C-1), 105.27d (C-2'), 140.16 C-3'), 146.88 (C-4'), 141.26 (C-5'), 106.69d (C-6'), 60.23q, 60.77q, 60.98q, 60.61q (4-OcH₃), 102.42t (-ocH₂O).

ALKALINE DEGRADATION OF 1: A solution of (40 mg) in 50% KOH (5ml) and EtOH (5 ml) were refluxed for 24 hrs. The reaction mixture was cooled and acidified by 30% HCl. The product were separated out and extracted with solvent ether. The ethereal layer was washed with H₂O and separated into two parts. The H₂O phase was acidified with 30% H₂SO₄ and extracted with Et₂O. The ethereal solution was dried and evaporated to yield colourless solid. After crystallization from MeOH gave la, 3-methoxy 4,5- methylenedioxy benzoic acid as colourless crystals (15mg) m.p. 207° (lit 206.7%); uv MeOH max nm: 269; 1H -nmr ($CDCl_3$): 3.94 (s, oMe), 6.07 (s, OcH₂O), 7.25 and 7.40 (d, J = 1.5 Hz, 2-and 6-H). ir ($CHCl_3\text{cm}^{-1}$); 3200-3540 and 1680 (Ar COOH), 1600 (Aromatics); MS m/e 196 (M^+ 100%) (Calc. for $C_9H_8O_5$ 196.037) 181 (M-Me). The ethereal layer was treated with 10% NaHCO₃ solution and the aqueous part on acidification gave a bright yellow compound identified as 2,6-dihydroxy 3,4-dimethoxy (1'-methoxy) acetophenone MS m/e 242 $C_{11}H_{14}O_6$ (M^+ 100) lb.



KOH FUSION

1b**1a****ACKNOWLEDGEMENTS**

Thanks are due to Director, Central Drug Research Institute, Lucknow, for recording various spectra.

REFERENCES

1. Wealth of India " A dictionary of Raw Materials and Industrial Products", C.S.I.R. Publication New Delhi, India, II, p. 382 (1950)
2. H. Werner, V.G. Seringlam, B. Keril, H. Wagner, Phytochemistry, 19, 669 (1980).
3. T.J. Mabry, K.R. Markham and M.B. Thomas, "The Systematic Identification of Flavonoids", Springer verlag, New York, 52 (1970)
4. S. Zeisels, Montash Chem. , 6, 989 (1983).
5. F. Dallacker and R. Sluysmans, Monatsh Chem. , 100, 560 (1969)