P: ISSN NO.: 2394-0344 RNI No.UPBIL/2016/67980 VOL-6* ISSUE-9*December-2021
E: ISSN NO.: 2455-0817 *Remarking An Analisation*

A Convenient Synthesis and Structural Elucidation of 3,5,3',4'-Tetrahydroxy-6,7,-Dimethoxyflavone (Eupatilin)

Paper Submission: 01/12/2021, Date of Acceptance: 10/12/2021, Date of Publication: 11/12/2021

Mridula Verma
Associate Professor,
Dept. of Chemistry,
M.M.H. College,
Ghaziabad, U.P., India

Abstract

The pharmacological benefits of phytochemicals have always been a field of interest. Flavonoids are a class of naturally occurring coloured pigments with remarkable health benefits. The structure, chemical composition and groups attached decide the potency of biological benefits of various flavones. Eupatolitin is a comparatively less explored flavone in spite of showing remarkable medicinal importance. The pigments isolated from plant Eupatorium ligustrinum on isolation, analysis, synthesis and structural elucidation proved to be Eupatolitin.

Key Words : Flavones, Flavonoids, Polyphenols, Antioxidants.

Introduction

The health benefits of naturally occurring substances have always been the field of interest for pharmacologists. Flavonoids are a group of bioactive compounds which are extensively found in foodstuffs of plant origin. Flavonoids in the broad sense of the term are virtually universal plant pigments, water soluble in nature and add colour to the flowers, fruits and some kind of leaves. These are considered as secondary metabolites in plants and fungus.

Review of Literature

Flavonols and flavones are plant-derived polyphenolic phytochemicals occurring ubiquitously in plants having a variety of biological effects both in vitro and in vivo[1]. These are also known as anthoxanthins, occurring either in free state or as glycosides. The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure. The configuration, substitution, and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability. There has been increasing interest in the research on flavonoids from plant sources because of their versatile health benefits reported in various epidemiological studies[2]. Oxidative stress is supposed to be the major cause of metabolic diseases and flavones show a positive effect on such disorders[3].Oxidative stress plays a key role in Dementia. The flavonoids present in wine are powerful antioxidants proved to play a protective role in such neuro disorders. Flavonoids possess many biochemical properties such as antioxidant, anti-proliferative, anti-tumor, anti-microbial, estrogenic, acetyl cholinesterase, anti-inflammatory activities and are also used in cancer, cardiovascular diseases, neurodegenerative disorders etc. Recent studies have shown the positive effects of flavones on diseases related to oxidative stress. Studies show that the consumption of Flavonoid rich foods can beneficially influence normal cognitive function and inhibit the development of Alzheimer diseases[4]. Intake of antioxidant flavonoids has been inversely related to the risk of incidence of Dementia. More than 4000 varieties of flavonoids have been identified, many of which are responsible for the attractive colours of flowers, fruits, and leaves. The best described property of almost every group of flavonoids is their capacity to act as antioxidants. Thus flavonoids found in animals are of plant origin rather than being biosynthesized in situ.Flavonoids found in the highest amounts in the human diet include the soy isoflavones, flavonols and the flavones. Although most fruits and some legumes contain catechins, the levels vary from 4.5 to 610 mg/kg.

The phytochemists found an interesting field of study with the discovery of the low cardiovascular mortality rate in mediterranean population, commonly known as French paradox. The significant biological activity of flavonoids present in red wine have been reported[5].
Cotelle et al[6] studied the role of hydroxyl groups on the biological activities of phenols by synthesizing 10 polyhydroxy flavones with varied substitution patterns and investigated these compounds as radical scavengers. The flavones and related compounds like catechins seem to be the most powerful flavonoids for protecting the body against various free radicals and

reactive oxygen species. The flavonoids scavenge hydroxy (˙OH) radicals generated by UV photolysis of H_2O_2. This capacity is increased as the number of hydroxyl groups increases. The flavonoid antioxidants function as free radical scavengers by rapid donation of a hydrogen atom to radicals. In general the radical scavenging activity of flavonoids depends on the molecular structure and the substitution pattern of hydroxyl groups. Due to rational abundance and significantly high range of biological activities of flavones, their structure activity relationships have generated interest among medicinal chemists.

Aim of the Study

Synthesis and structural determination of 3,5,3',4' -tetrahydroxy-6,7-dimethoxyflavone.

Structure

Flavones are class of flavonoids based on the backbone of 2-phenyl chromen-4-one and are coloured in nature. The common flavones include apigenin, luteolin, tangeritin, chrysin and 6- hydroxy flavone. The basic unit of flavones and flavonols is γ-pyrone present as benzo-γ-pyrone (chromone) A, as backbone structure. Depending upon the substitution on benzo- γ -pyrone skeleton the nature of flavone changes. In nature flavones and flavonols occur as glycosides and yield glucose on rhamnose unit along with a sugar free unit (aglycon) on hydrolysis. This aglycon portion is known as anthoxanthin (flavone) or flavonol.

The therapeutic properties of flavonoids are due to their polyphenolic nature and depend upon the structure of the molecule. The arrangement of hydroxyl groups in the basic backbone skeleton decide the nature of the compound (flavonoids)

The present communication is about a simple and convenient synthesis of a naturally occurring flavone Eupatolitin in the plants. Eupalitin is one of the pharmacologically active ingredients of DA-9601 and studies show the function of Eupatolitin in vitro are attributed to the induction of apoptosis in many cell types[7,8].

Chemistry of Eupatolitin

Eupalitin is found to be an isomer of axillarin (1), has been proposed its constitutional tetrahy droxy flavone substituted by hydroxyl groups at positions 3,5,3' and 4' and methoxy groups at positions 6 and 7 respectively(2). Eupalitin and Eupatolitin glycosides have been identified in plant Ipomopsis aggregata[9]. The chemical examination of Eupatorium ligustrinum reported by Quijano et al.[10] showed the presence of two naturally occurring pigments one with molecular formula $C_{17}H_{14}O_7$ (m.p. 289-92^{0}c) named Eupalitin whereas, the second pigment with molecular formula $C_{17}H_{14}O_8$ (m.p. 285-87^{0}c) was named Eupatolitin. On the basis of physical studies, colour reactions, spectral studies and derivatives the pigment Eupalitin is found to be 3,5,4'-trihydroxy-6,7-dimethoxy flavone(3) whereas, Eupatolitin was given its constitution as 3,5,3',4'-tetrahydroxy-6,7-dimethoxy flavone(2). In this communication a very simple and convenient synthetic route for the synthesis followed by structural elucidation of Eupatolitin is reported. This synthetic route involved the preparation of an important intermediate 3,6,7-trimethoxy-5,3',4'-trihydroxy flavone(4) which incidentally also provided a convenient synthesis for another polyphenolic pigment , cyanostephylla-B.

Synthesis of Eupatolitin

TheIUPACname2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-6,7-dimethoxychromen-4-one. The present synthesis of Eupatolitin involved the selective demethoxylation of 3,6,7-trimethoxy-5,3',4'-trihydroxyflavone(4)to obtain 3,5,3',4'-tetrahydroxy-6,7-dimethoxy flavone(2). The selective demethylation of C_3 methoxyl in presence of C_5 hydroxyl by using hydrobromic acid in acetic acid has been reported[11]. The demethylated product of compound (4) was characterized as 3,5,3',4'-tetrahydroxy-6,7-dimethoxy flavone(2). The Steps of analysis are as follows.
The isolated product followed by hydrolysis gave the compound with molecular formula $C_{17}H_{14}O_8$(2). This on acetylation formed a tetraacetate $C_{25}H_{22}O_{12}$(5) supporting the presence of 4-hydroxyl groups on(2) . The alkaline hydrolysis leading to the fission of the molecule yielded compound(6) called protocatechuic acid suggesting the two hydroxyl groups at C_3 and C_4 positions of the side phenyl ring of the γ pyrone unit. The negative Asahina-Incubus test again confirms the presence of a hydroxyl function at the C_3 position of the side ring. Further the negative Bargellini test confirms the absence of a 5,6,7-trihydroxy system in the molecule.

Experimental

The 3,6,7 - Trimethoxy-5,3',4'- trihydroxy flavone (4) (1.0gm) was treated with hydrobromic acid in acetic acid (30ml) and the resulting reaction mixture was heated on a water bath for 3hrs. The reaction mixture then cooled and poured over crushed ice with constant stirring. The product then extracted with ethyl acetate, washed with 10% sodium bicarbonate solution and finally with water. The ethyl acetate was dried over anhydrous sodium sulphate. On removal of the solvent, a yellow residue was obtained which crystallised from methanol to give 3,5,3',4'-tetrahydroxy-6,7 - dimethoxy flavone(2) as yellow needles (0.7gm), m.p.285-86^{0}c, with molecular formula $C_{17}H_{14}O_8$. It gave green coloration with ethanolic ferric chloride.

Results and discussions

Based on the above considerations demethylated product was found to be the required 3,5,3',4'-tetrahydroxy-6,7 - dimethoxy flavone(2) . Synthetic 3,5,3',4'-tetrahydroxy-6,7 - dimethoxy flavone and its acetate (5), methyl ether(7) and ethyl ether(8) were found to agree with the acetate, methyl ether and ethyl ether of the isolated compound and support the study and structure of Eupatolitin.

Compound 2

UV SPECTRUM (λmax)

CH_3 OH	262, 276(sh), 372nm.
CH_3 OH + $AlCl_3$	280, 316(sh), 448 nm.
CH_3 OH + $AlCl_3$+HCl	272, 298 (sh), 382 nm.
CH_3 OH +NaOAc	262, 316 (sh), 402 nm.
CH_3 OH +NaOAc+H_3BO_3	262, 316 (sh), 384 nm.

NMR SPECTRAL DATA RECORDED IN $CDCl_3$

δ3.90- δ3.96 (6H, m, 2X-OCH_3,), δ6.84 (1H, s, C_8-H), δ 7.12(1H, d, J=9Hz, C_5-H), δ7.80 (2H, m, C_2 -H and C_6 -H).

Compound 5

3,5,3',4'-TETRAACETOXY-6,7-DIMETHOXYFLAVONE (5):

3,5,3', 4'-Tetrahydroxy-6,7-dimethoxyflavone (2) (100 mg) was treated with acetic anhydride (lml) and pyridine (0.5 ml) and the resulting solution was heated on a boiling water-bath for 2 hrs. It was then cooled at room temperature for 1 hr. The reaction product was treated with crushed-ice and stirred well to decompose the excess of acetic anhydride and then extracted with ether. The ether extract was treated with sodium bicarbonate (5%), washed with dilute hydrochloric acid, then with water and finally dried over anhydrous sodium sulphate. Removal of the solvent gave the required 3,5,3', 4'-tetraacetoxy-6,7- dimethoxyflavone(5) which crystallised from ethanol as colourless needles , m.p. 198°c, $C_{25}H_{22}O_{12}$.

UV SPECTRUM (λmax)

CH_3 OH	262, 276(sh), 372nm.
CH_3 OH + $AlCl_3$	280, 316(sh), 448 nm.
CH_3 OH + $AlCl_3$+HCl	272, 298 (sh), 382 nm.
CH_3 OH +NaOAc	262, 316 (sh), 402 nm.

CH_3 OH +NaOAc+H_3BO_3 262, 316 (sh), 384 nm.

NMR SPECTRAL DATA RECORDED IN $CDCl_3$

δ2.30- δ2.40 (12H, m, 4X-$OCOCH_3$,), δ3.98- δ4.04 (6H, m, 2X-OCH_3), δ6.98 (1H, s, C_8-H), δ7.34 (1H, d, J=9Hz, C_5-H), δ7.82 (2H, m, C_2- H and C_6-H).

Compound 7

3,5,6,7,3',4'-HEXAMETHOXYFLAVONE (7)

3,5,3', 4'-Tetrahydroxy-6,7-dimethoxyflavone (2) (50 mg) was treated with dimethyl sulphate (0.1 ml), potassium carbonate (500 mg) and dry acetone (100 ml), and the resulting mixture was heated under reflux for 5 hrs. The inorganic salts were filtered out, washed with hot acetone and the solvent was then removed from the combined filtrate under reduced pressure. The reaction product was treated with ice cold water, extracted with ether and the ether extract was dried over anhydrous sodium sulphate. Removal of the solvent gave a residue that crystallised from aqueous ethanol to give required methylated product 3,5,6,7,3',4'-hexamethoxyflavone (7) as colourless needles, m.p.145-46^{0}c, $C_{21}H_{22}O_8$.

NMR SPECTRAL DATA RECORDED IN $CDCl_3$

δ3.85 - δ4.05 (18H, m, 6X-OCH_3,), δ6.65 (1H, s, C_8-H), δ6.81.- δ6.91(1H, d, J=9Hz, C_5-H), δ7.42 - δ7.62 (2H, m, C_2-H and C_6-H)

Compound 8

3,5,3',4'-TETRAETHOXY-6,7-DIMETHOXYFLAVONE (8)

3,5,3', 4'-Tetrahydroxy-6,7-dimethoxyflavone (2) (70 mg) was treated with ethyl iodide (2ml), potassium carbonate (500 mg) in dry acetone (100 ml) and the resulting reaction mixture was heated under reflux for 40 hrs. The inorganic salts were filtered out, washed with hot acetone, and the solvent was then removed from the combined filtrate under reduced pressure. The reaction product was treated with water, extracted with ether and the ether extract was dried over anhydrous sodium sulphate. Removal of the solvent gave a residue that crystallised from aqueous ethanol to give 3,5,3',4'-tetraethoxy-6 ,7-dimethoxy flavone (8) as colourless needles, m.p. 126- 27^{0}c, $C_{25}H_{30}O_8$.

NMR SPECTRAL DATA RECORDED IN $CDCl_3$

δ 1.25- δ1.60(12H, m, 4XOCH_2CH_3,), δ3.96- δ4.00(6H, m, 2X-OCH_3), δ4.20- δ4.30 (8H,m, 4X-OCH_2CH_3), δ6.68 (1H, S,C_8-H), δ6.64- δ6.74 (1H,d,J=9Hz, C_5-H), δ7.42- δ7.60(2H, m, C_2-H and C_6-H).

Conclusion

The isolated compound is identified as Eupatolitin. The structure has been elucidated by preparing different derivatives and analyzing by the application of spectroscopic techniques. Results are in agreement with the given structure.

References

1. *Gregory L Hostetler, Robin A Ralston, StevenJ Schwartz. Advances in nutrition 8(3),423-435 (2017).*
2. *Owen L Wood man, Elsa CH chan. Clinical and Experimental Pharmacology and Physiology 31(11), 786-790, (2004).*
3. *D. Commenges, V. Scotet, S.Renaud, H.Jacqmin-Gadda, P.Barberger-Gateau & J.F.Dartigues. European Journal of Epidemiolgy, 16, 357-363(2000).*
4. *Robert J. Williams & Jerempy P.E. Spencer Free Redical Biology and Medicine, Vol.52, Issue1, 35-45, (2012).*
5. *Robert J Nijveldt, Els van Nood, Danny EC van HoornPetra G Boelens, Klaske van Norren, Paul AM van Leeuwed . The American Journal of Clinical Nutrition, vol. 74, Issue 4, 418-425, October (200l),*
6. *N. Cotelle, JL Bernier, JP Henichart, JP Catteau, E Gaydou, J C Wallet. Free radical Biology and Medicine, 13 (3), 211-219 (1992).*
7. *H.J.Seo and Y.J. Surth, Mutat.Res. ,496, 191-198 (2001).*

8. *M.J.Kim, D.H.Kim, H.K.Na, T.Y.Oh, C.Y.Shin and D.P. Y.J. Surth. J.Environ. Pathol. Toxicol. Oncol., 24, 261-269 (2005).*
9. *Dale M.Smith, C.W.Glennie, J.Harborne. Chemistry Phytochemistry (1971).*
10. *L. Quijano, F. Malanco and T.Rios, Tetrahedron 26, 2811 (1970).*
11. *D.K. Bhardwaj, S. Neelakantan and T.R. Seshadri, Indian J. Chem, 4, 417, (1966).*