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Isolation and Structure Elucidation of Olea Europaea

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Research Article

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Abstract

The present study comprises on isolation and structure elucidation of four known compounds for the first time from medicinally important species Olea europaea. The powder material of shade-dried plant of O. europaea was extracted several times in Ethanol. This extract was concentrated with the help of rota-vapour to gain the brownish thick gummy crude. The gummy residue was fractionated into four different fractions on the basis of their polarities i.e. n-hexane, CH2Cl2, EtOAc and Ethanol. From these fractions, the EtOAc fraction was further carried out to perform the process of column chromatography over silica gel with increasing order of different organic solvents system to obtain different compounds. TLC cards were used to check purify of the isolated compounds in different solvents system. The structures were confirmed through modern spectroscopic techniques as, 1H NMR, 13C NMR, IR, UV and EIMS.

Keywords: *Olea europaea*; Isolation; Characterization; Compound 1-4

Introduction

Natural products can be described as products of natural genesis [1]. The term natural product has been differentiated into both limited and controversial points. Simply, the term "natural product" is a small molecule which is formed by a biological source. The natural products research has focus on the chemical properties, bio-synthesis and biological functions of secondary metabolites as a central theme of examine and bordering chemistry and biology [2]. So, simple definition is: A living organism produced a chemical substance; a term normally used in reference to chemical substances found in nature which have characteristic pharmacological impacts [3]. Natural products have an important role in the scientific field of pharmacognosy through its study of identification, isolation and characterization. The American Society of Pharmacognosy characterizes pharmacognosy as "The investigation of natural molecules (normally secondary metabolites) that are helpful for their therapeutic, biological, gustatory, or other utilitarian properties. During the investigation of all biological kingdoms, especially marine invertebrates, plants, fungi and bacteria, these natural species are found as a source of the compounds". In general the word "natural product" is same as "secondary metabolite" [2].

The secondary metabolites are obtained from primary metabolites for example carbohydrates, amino acids and lipids and it can be ordered into few chemical groups like alkaloids, phenyl propanoids, polyketides, tepenoids and phenolics [4-6].

Olea europaea is called Olive commonly [7]. And it exists in the *Olea* genus of the *Oleaceae* family [8], of Angiosperm (flowering plants) [9]. It is subjected to the Mediterranean

regions as from Syria to the south end of the Caspian Sea, Asia and northern Iraq. It is scattered between 30° to 45° latitudes in the northern and southern hemispheres and from sea-level to 900 m altitude. But it's also growth occur at 1,000-3,150 ft altitude. Sunny day is needed for the production of Olive fruit whereas slight winter chill is also needed. Fruit vernalization period is 7-12 weeks below 8°C. Below −10°C the tree will be killed due to frostiness. The chill coolness may harm the flowers and fresh shoots while the ripping fruits may also be harmed in the end of the autumn. Shoots and flowers can best grow under the temperatures between 18-22°C. The flowers are always destroyed during the temperature above 30°C in spring while the tree may serve even in the higher temperature throughout summer [10]. It can found in well-drained soil of pH below 8.5 [11]. It remains green throughout the year, having length of 6-9 m which is equally or sometime slightly less spread. The truck is gray in color massively and bumpy contorted. Mostly trees have spreading crowns and rounded. But in Italy, the trees are cylindrical tall. Leaves are silver green in color having 7.5 cm size, linear, narrow and opposite with sharp tips and intact margins. Flowers are small creamy white in color. One year old tree has axillary clusters of 15 flowers which bloom in spring. Juvenile period of seeds are long. Most flowers experience pistil premature birth, leaving just a single to twice fruits for each axil at harvest. Olive fruit is edible having drupes, 4 cm in size, and green in color during end of the summer and turns into black in color on maturity. Its fruit falls on ripping. Olive's maturation period is 6-8 months. When oil content reaches 20-30% in olive then it is plucked while table olive is plucked earlier on firming [12]. It have 46 chromosomes i.e. 2n = 2x = 46 and it is a diploid specie [13].

Edible fruit is only produced by *Olea europaea* specie. No fruit is born on the olive tree for the initial 10-12 years. The fruit growth is slow during the months of spring and summer. But in the month of autumn it grows faster due to water content. The fruit weight is 1-15g individually. Oil deposition occur in the fruit during the month of August and in autumn it increases while November to January the fruit turns into black color and oil reaches on its peaks [11]. The mature seed is enclosed with a thin coat that covers the starch filled endosperm. It also surrounds the flat, tapering leaf, roots and stems. Seeds shape and size varies with cultivar. The best development of seed occurs in the month of July to September [14].

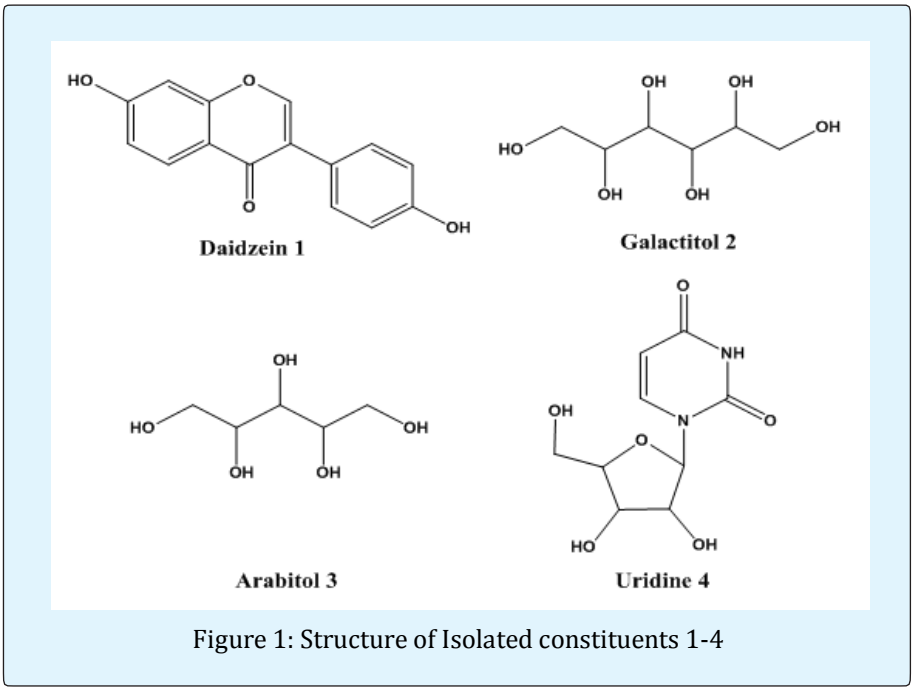

Figure 1: Structure of Isolated constituents 1-4

It is economically well known specie having important crop, ornamental [15]. Commercial importance of this specie is for food, cosmetics, lumber and also medicinal products. It is also used as folk medicine for the treatment of neurotic and chronic lung disorder and also for treating neurological disorder, insomnia, heart failure, icterus, tussis, and have wound healing actions. It is also involved in the treatment of various types of acute and chronic inflammatory diseases as a herbal medicine [8,16].

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Results and Discussion

Ethyl acetate fraction was subjected to column chromatography using silica gel. The column was run with increasing polarity order of different organic solvents as, *n*-hex, $CHCl_3$, EtOAc and EtOH to obtain compound 1-4. Purification was done using TLC in different solvent systems.

Daidzein (1) was identified by various analytical techniques and chemical-test. Compound **1** obtained as a pale yellow colored crystalline solid with melting point of 315-320°C. Its molecular formula was determined as $C_{15}H_{10}O_4$ by an ion peak at m/z 253.1 [M-1] $^+$.In the IR spectrum, absorption bands were obtained at 3456 cm^{-1} (free phenolic OH), 1601 cm^{-1} (C=C). The compound **1** showed negative Shinoda test which indicated the presence of daidzeinisoflavone. Isolated compound was identical to those spectral data and chemical properties which are reported in earlier for daidzein [17-19].

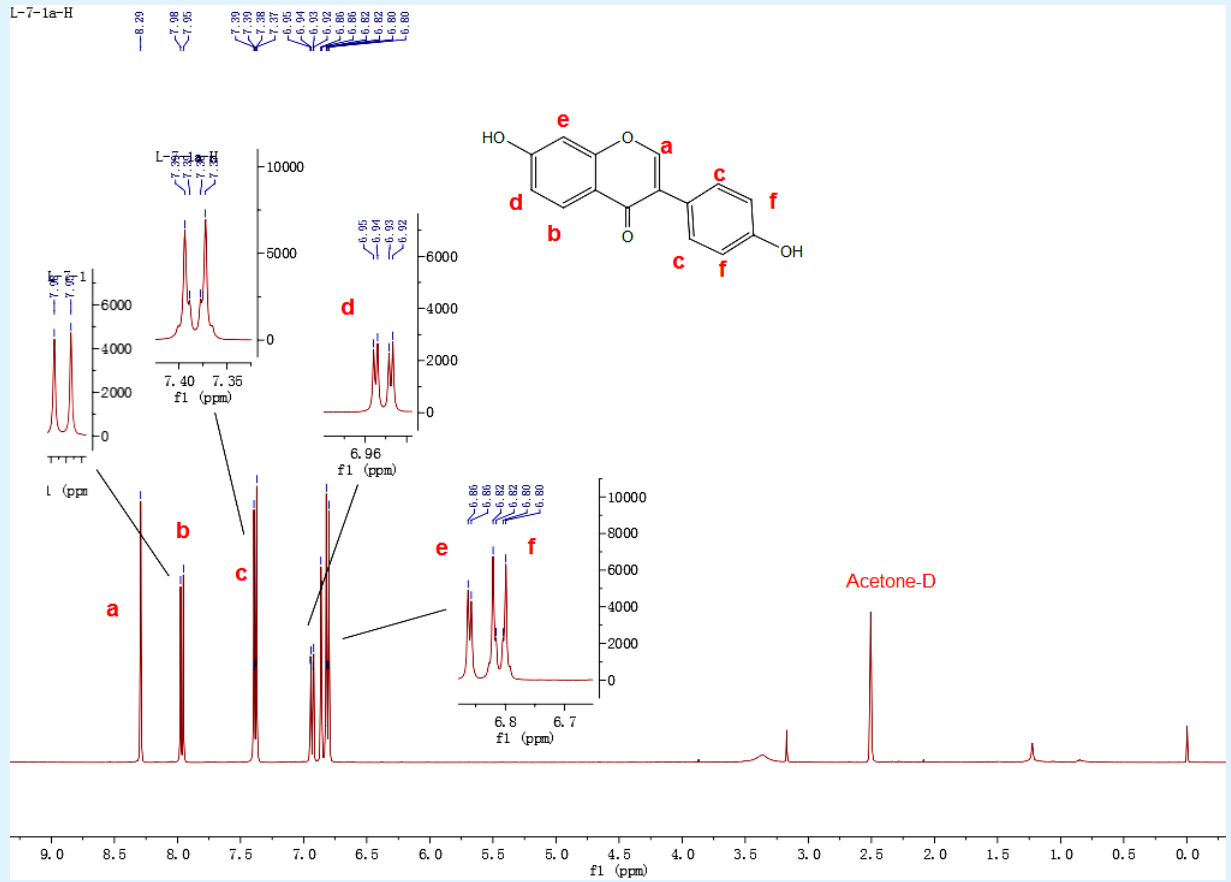

Figure 2: HNMR spectrum of isolated compound Daidzein (1).

Galactitol (2) was determined by different analytical methods and chemical test. Compound **2** was found as amorphous solid with melting point of 189- 120°C. Its molecular formula was determined as $C_6H_{14}O_6$ by an ion peak at m/z 182.172 [M-1] $^+$. In the IR spectra, resonating signals at 3600 cm^{-1} shows the free alcohic OH group.^{1}H-NMR and ^{13}C-NMR data of galactitol compound is shown in experimental section. In thin layer chromatography (TLC) study, compound illustrated single band on silica gel plate at Rf value 0.3 in solvent system n-Hexane: Methanol (95:5). Galactitol is a known compoundbecause its spectral data and chemical properties are similar as reported earlier [20,21].

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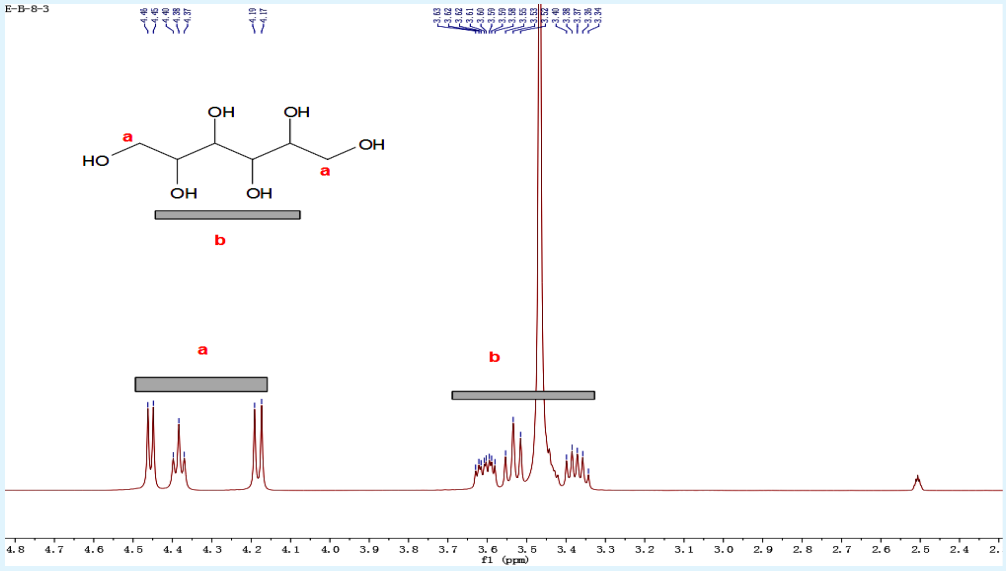

Figure 3: HNMR spectrum of isolated compound Galactitol (2).

Arabitol (3) compound was identified by analytical methods along with chemical test. Compound**3** was isolated as colorless amorphous solid with melting point of 100 - 103°C. Its molecular formula was calculated as $C_5H_{12}O_5$ by an ion peak at m/z 152.145 $[M-1]^+$. The IR vibrational signals are found at 3624 cm^{-1} which indicated that compound **3** has free alcoholic OH group. In thin layer chromatography (TLC) analysis, compound showed single band on silica gel plate at Rf value 0.4 in solvent system n-Hexane: Methanol (90:10). Spectral data and chemical properties of obtained compound were considered to those reported in earlier for arabitol [22].

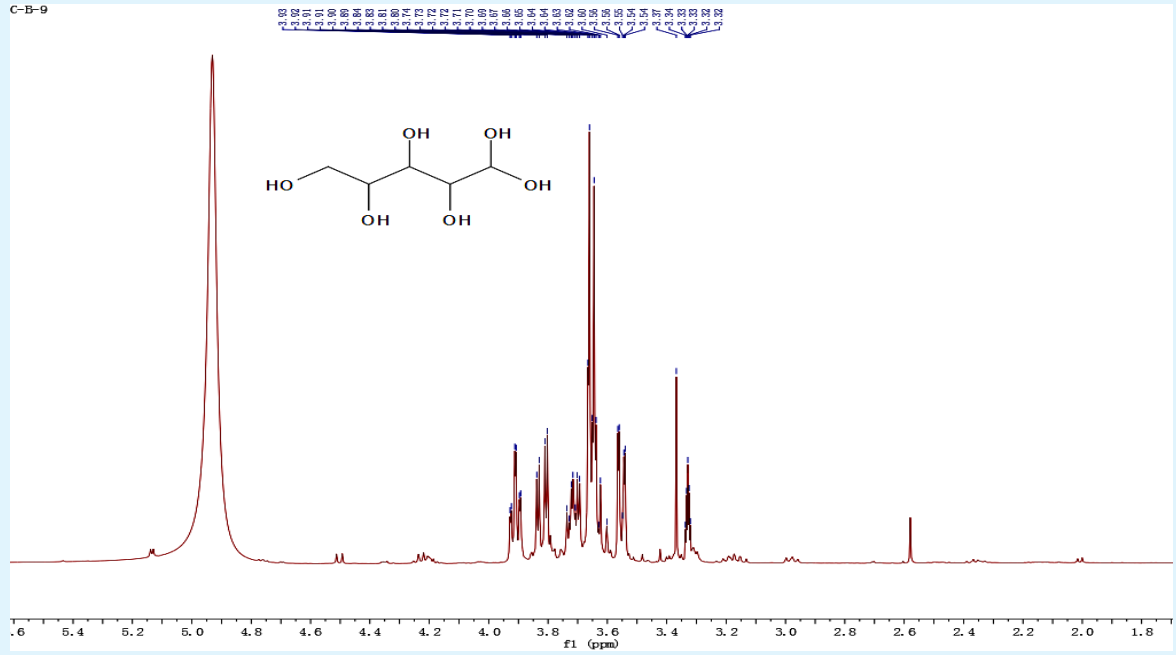

Figure 4: HNMR spectrum of isolated compound Arabitol (3).

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Uridine (4) was obtained by different chemical test and analytical methods. Compound 4 was isolated as colorless crystalline solid with melting point of 163-165 °C. With the help of HREI-MS, its chemical formula was resolute as $C_9H_{12}N_2O_6$ by an ion peak at m/z 244 [M-1] $^+$. In the IR spectrum, different absorption bands were set up at 3640 cm^{-1} (free alcoholic OH), 1601 cm^{-1} (C=O), 3300 cm^{-1} (sec amine). In thin layer chromatography (TLC) analysis, compound 4 showed Rf value 0.5 in solvent system n-Hexane: Methanol (80:20) in the form of single band on silica gel plate. Uridine has identical spectral data and chemical properties to those reported in earlier for uridine [23-26].

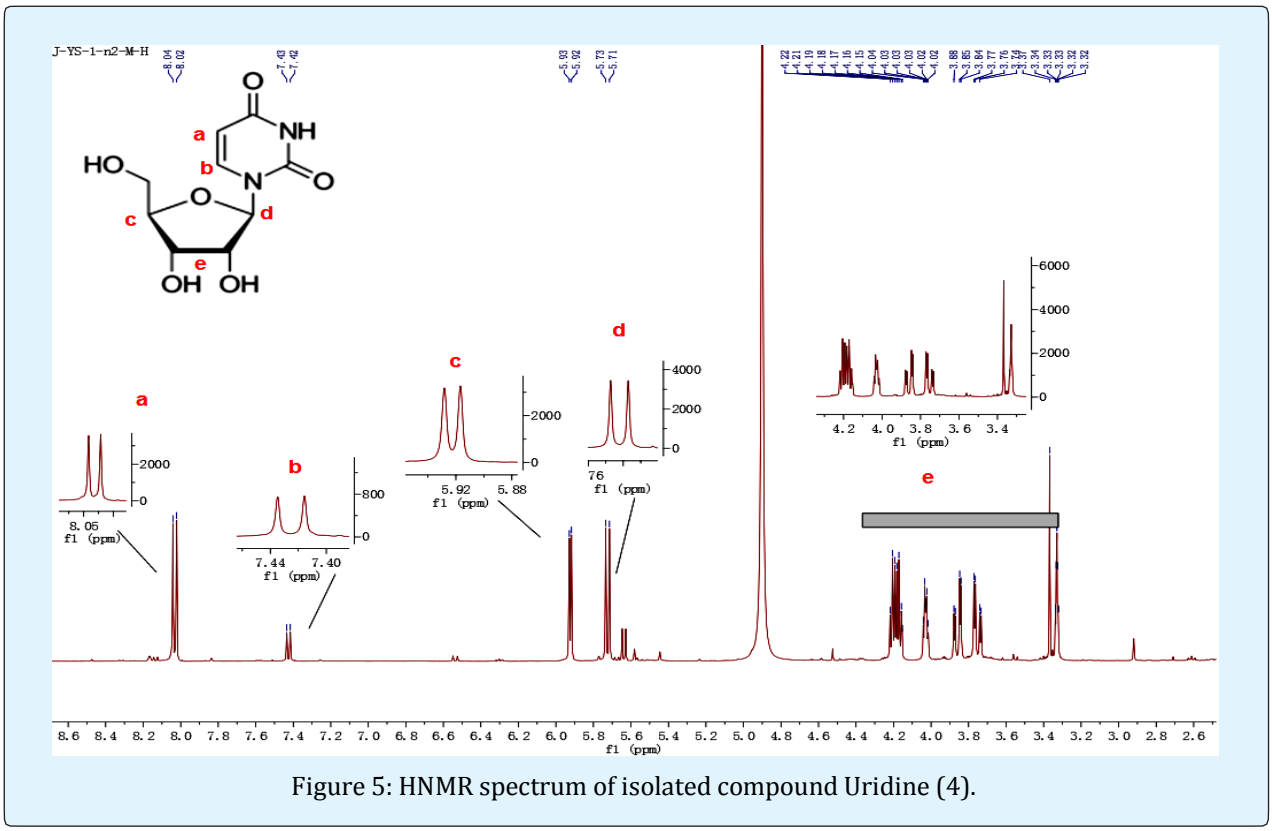

Figure 5: HNMR spectrum of isolated compound Uridine (4).

Experimental Section

Plant Material

The plant material of *Olea europaea* was collected from Karak, D.I. Khan, KPK, Pakistan. Prof. Dr. Sadiq Khan, Mufti Mehmood University of Agriculture D.I.K has confirmed the identification of *Olea europaea* plant.

Extraction and Isolation

The branches and leaves of plant was shade dried for 40 days. The powdered material of shade dried plant of *Olea europaea* (3 kg) was extracted 3 times in Ethanol. This extract was concentrated with the help of rota-vapour to gain the brownish thick gummy crude (98 g). Then this crude was placed in Ethanol and separated into different fractions of *n*-hex (F-1, 15 g), $CHCl_3$ (F-2, 17 g), EtOAc (F-3, 21 g) and EtOH (F-4, 15 g).

From these fractions, the EtOAc (F-3, 21 g) fraction was further carried out to perform the process of column chromatography over silica gel with increasing order of different organic solvents polarity like *n*-hex, $CHCl_3$, EtOAc, and EtOH, to obtain different compounds. TLC cards were used to purify the compounds in different solvents system.

Daidzein (1)

Pale yellow color crystalline (Purity >99%); IR Spectrum: 1601 cm^{-1}, 3456 cm^{-1}; HR-EMIS m/z 253.1 [M-1] $^+$, (for $C_{15}H_{10}O_4$); ^{1}H-NMR: CH_3OD, 400MHz δ (ppm): δ=6.86 (1H, s, H-1), 6.95 (1H, d, H-3), 7.98 (1H, d, H-4), 8.29 (1H, s, H-7), 7.39 (1H, d, H-2′), 6.82 (1H, d, H-3′), 6.80 (1H, d, H-5′), 6.86 (1H, d, H-6′); ^{13}C-NMR: CH_3OD, 100MHzδ (ppm): δ=102.64 (C-1), 163.05 (C-2), 115.39 (C-3), 127.73 (C-4),

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176.14 (C-5), 123.0 (C-6), 153.62 (C-7), 157.89 (C-8), 117.03 (C-9), 123.92 (C-1′), 130.53 (C-2′), 115.61 (C-3′), 157.61 (C-4′), 115.61 (C-5′), 130.53 (C-6′).

Galactitol (2)

Colorless amorphous solid (Purity >99%)**;** IR Spectrum: 3600 cm^{-1}; HR-EMIS m/z 182.172 [M-1] $^{+}$, (for $C_6H_{14}O_6$); ^{1}H-NMR: CD_3OD, 400MHzδ (ppm): δ=4.46 (2H, d, H-1), 3.63 (1H, m, H-2), 3.60 (1H, t, H-3), 3.60 (1H, t, H-4), 3.63 (1H, m, H-5), 4.46 (2H, d H-6); ^{13}C-NMR: CD_3OD, 100MHzδ (ppm): δ=64.30 (C-1), 70.07 (C-2), 71.72 (C-3 & C-4), 70.07 (C-5), 64.30 (C-6).

Arabitol (3)

Colorless amorphous solid (Purity >99%); IR Spectrum: 3624 cm^{-1}; HR-EMIS m/z 2.145 [M-1] $^{+}$, (for $C_5H_{12}O_5$); ^{1}H-NMR: CD_3OD, 400MHz δ (ppm): δ= 3.80(2H, d, H-1), 3.91(1H, m, H-2), 3.60(1H, t, H-3), 3.71(1H, m, H-4), 3.80(1H, d, H-5); ^{13}C-NMR: CD_3OD, 100MHzδ (ppm): δ=63.90 (C-1), 71.0 (C-2), 63.98 (C-3), 73.0 (C-4), 7.98 (C-5).

Uridine (4)

Colorless crystalline solid (Purity >98%); IR Spectrum: 1601 cm-1, 3300 cm-1, 3640 cm-1; HR-EMIS m/z 244 [M-1] +, (for C9H12N2O6); ^{1}H-NMR: CD3OD, 400MHz δ (ppm): δ=8.04 (1H, d, H-2), 7.43 (1H, d, H-3), 5.73 (1H, d, H-5), 4.22 (1H, t, H-6), 4.18 (1H, t, H-7), 5.93 (1H, q, H-8), 3.84 (2H, d, H-9); 13C-NMR: CD3OD, 100MHz δ (ppm): δ=164.80 (C-1), 101.28 (C-2), 141.34 (C-3), 151.08 (C-4), 89.27 (C-5), 69.91 (C-6) 74.32 (C-7), 84.96 (C-8), 60.88 (C-9).

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