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New furocarbazole alkaloids from Lonicera quinquelocularis

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Two new furocarbazole alkaloids, 3-formyl-6,7-dimethoxy-furo[1,2]carbazole (**1**) and methyl-6,7-dimethoxy-furo[1,2]carbazole-3-carboxylate (**2**), along with two known carbazole alkaloids, 3-formyl-2-hydroxy-7-methoxycarbazole (**3**) and methyl 2,7-dimethoxycarbazole-3-carboxylate (**4**) were isolated from the ethyl acetate soluble fraction of Lonicera quinquelocularis. Their structures were established on the basis of spectroscopic analysis.

Keywords: Lonicera quinquelocularis, Caprifoliaceae, furocarbazole alkaloids.

1. Introduction

The genus Lonicera belongs to the family Caprifoliaceae comprises of 12 genera and 450 species, found in temperate region of Northern Hemisphere. Plants of this genus are used for the treatment of acute fever, headache, respiratory infections [Houghton et al., 1993], antibacterial, antioxidant, cytoprotective, hepatoprotective, antiviral, anti-tumour and anti-inflammatory activities [Puupponen-Pimia et al., 2001; Kahkonen et al., 2001; Shi et al., 1999; Wang et al., 2009, & Yoo et al., 2008]. Previous literature of this plant showed the isolation of various phytoconstituents

such as iridoids, bisiridoids, sulfur containing monoterpenoids, alkaloidal glycosides, triterpenoids, saponins, coumarin glycosides and flavone glycosides [Machida et al., 1995; Bailleul et al., 1991; Souzu and Mitsuhashi, 1969, & Souzu and Mitsuhashi, 1970]. Lonicera quinquelocularis belongs to this genus mostly found in dry sunny places between 750and 3000 m in many countries of Asia. Previous phytochemical study on this plant has found the isolation of triterpenoids, lonicerin, loganins, coumarins, iridoide glycosides, phthalates and benzoates [Kumar et al., 2000; Ali et al., 2013; Khan et al., 2014a, & Khan et al., 2014b] from this plant. The diverse medicinal importance of genus Lonicera has prompted us to investigate the constituents of L.quinquelocularis. Herein, we reported the isolation and identification of two new furocarbazole alkaloids namely, **1** and **2** along with two known alkaloids **3** and **4** (Figure 1).

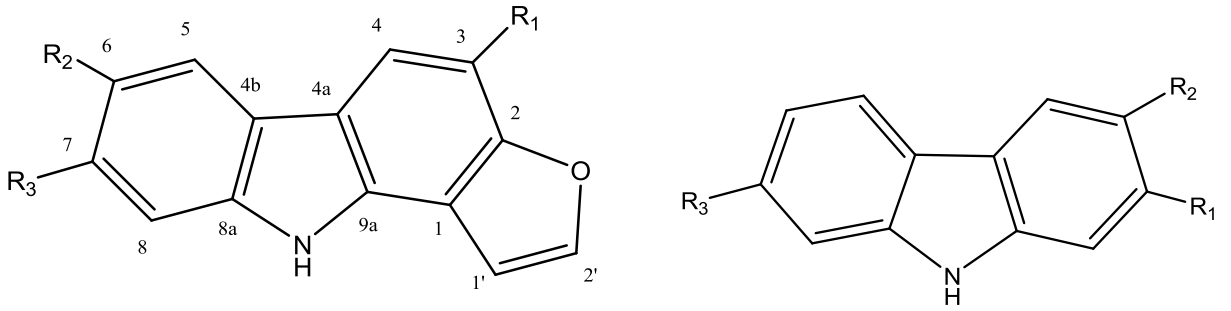

Figure 1. Structures of compounds 1-4

2. Results and Discussion

Compounds **3** and **4** were known compounds and were identified as 3-formyl-2-hydroxy-7-methoxycarbazole (**3**) [Chaichantipyuth et al., 1988;] and methyl 2,7-dimethoxycarbazole-3-carboxylate (**4**) [Wu et al., 1997].

Compound **1** was isolated as yellow crystals. The UV spectrum [λ_{max} 204 (4.10), 234(4.37), 245 (4.22), 278 (4.34), 285 (4.21), 332 (3.96) and 361 (3.90) nm] and the IR spectrum (υ_{max} at 965, 1190, 1510, 1578, 1631, 1658, 3263 and 3400 cm-1) indicated a 3-formyl furocarbazole framework. ^{1}H NMR spectrum (Figure S1) displayed signals at δ 8.54 (1H), 7.59 (1H) and 7.02 (1H) all singlet were characteristics of substituted aromatic A and C ring of the carbazole moiety. The peaks resonated at 7.83 (1H, d, J = 7.3 Hz) and 7.36 (1H, d, J = 7.3 Hz) each doublet (J = 7.4 Hz) were characteristics of furan ring fused to carbazole nucleus. The presence of ^{1}HNMR signal at 10.09 along with the ^{13}C NMR peak (Figure S2) at 187.56 gave assignment about the presence of aldehyde group which was further confirmed by the most deshielded signal at δ 8.54 assigned to H-4 due to the ortho-formyl group. Two singlets each for 3H found at δ 3.82 and 3.78 were assigned to two methoxy groups attached to the aromatic rings. The presence of a formyl group was further confirmed by the observation of a mass fragmentation ion at m/z 267 (M-CO). In addition, a mass spectral fragment at m/z 265 (M-OCH_3) and 235 (M-2OCH_3) suggested the presence of two aromatic methoxy groups. The HMBC correlations (Figure S5) were in conformity with the assigned structure of compound **1**. The data were in close agreement with the available literature (Ito and H. Furukawa, 1990).

The IR spectrum of compound **2** showed bands for NH (3440 cm^{-1}) and COOMe groups (1705 cm^{-1}), and for an aromatic system (1615, 1580, 1227, 1195, 1040, 815 and 728 cm^{-1}) indicating the carbazole framework. The methyl ester was indicated by a ^{1}H NMR (Figure S3) displayed singlet at δ 3.92 for the methoxy group and in the ^{13}C NMR spectrum (Figure S4), by a signal at δ 165.4 for the ester carbonyl group. The presence of a carbomethoxy functionality at the carbazole nucleus was additionally confirmed by the characteristic mass fragmentation at m/z 295 (M-OCH_3) and 265 (M-$COOCH_3$). Moreover, in ^{1}H NMR spectrum the presence of

aromatic methoxy groups (δ 3.80 and 3.75), the signals at δ 7.56 (s, 1H) and 7.04 (1H, s) for substituted carbazole framework and a deshielded H-4 singlet at δ 8.66, due to the proximity of the carbomethoxy function at C-3, were observed. The fused furan ring was indicted by the signals at δ 7.85 and 7.29 each one proton doublet (J = 7.6 Hz). The data coincide with the literature (Wu et al., 1997)

3. Experimental

3.1 General Experimental Procedures

Melting point was determined by using the Kofler hot-stage apparatus (Reichert, Vienna, Austria). Aluminium TLC plates (20 × 20, 0.5 mm thick) pre-coated with silica gel 60 F_{254} (0.2 mm layer thickness; E. Merck, Darmstadt, Germany) were used for TLC to check the purity of the compounds. Column chromatography (CC) was carried out using silica gel of 230-400 mesh (E. Merck). Preparative TLC glass plates (20 × 20, 2mm thick) pre-coated with silica gel 60 F_{254} (0.5 mm layer thickness; E. Merck) were used for the purification of semi-pure compounds. Ceric sulphate and potassium permanganate solutions were used as visualisation reagents. The UV spectra (λ_{max} nm) were recorded on Shimadzu UV-2700 spectrophotometer (Shimadzu, Kyoto, Japan) in EtOH. Mass Spectra was recorded on Bruker TOF Mass spectrometers (Billerica, MA, USA) using electrospray ionisation (ESI). The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 NMR spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C-NMR), using $CDCl_3$ as solvents. Further assignments were made by DEPT, COSY, HMQC and HMBC experiments.

3.2. Plant Material

The whole plant of L. quinquelocularis was collected from Bara Galli, Hazara division, District Mansehra. It was identified by Professor Dr. Manzoor Ahmad, Plant Taxonomist, Department of Botany, Government Degree College Abbotabad where a voucher specimen has been deposited in herbarium (Accession No. C-0013).

3.3 Extraction and Isolation

The shade dried whole plant of L. quinquelocularis (13 kg) was ground and extracted with ethanol at room temperature (3 X 25 L). The combined ethanolic extract was evaporated under reduced pressure to obtain a thick greenish gummy material (crude). It was suspended in water and successively partitioned with suitable solvents to yield n-hexane (151 g), chloroform (147 g), ethyl acetate (109 g) and n-butanol (53 g) soluble fractions, respectively.

The ethyl acetate soluble fraction was subjected to column chromatography over silica gel (70-230 mesh) eluting with n-hexane (100 %), n-hexane: EtOAc (1: 19–19:1), EtOAc (100 %), EtOAc:MeOH (1: 19–19:1), MeOH (100 %), in increasing order of polarity to obtain 13 fractions A-M.

The subfraction E gave two spots on TLC with good R_f value and hence were separated by preparative TLC eluted with n-hexane: CH_2Cl_2 (9:1-2: 8) to get the two spots separately which were placed in fume hood to get fine crystals of compound **1** and **2**.

The subfraction F on placing in fume hood spontaneously converted into fine crystals with traces of impurities which was washed with n-hexane: EtOAc (20: 1) till a single spot was obtained on TLC. This was again recrystallised using double distilled hexane to get fine and pure crystals which was confirmed as compound **3** by spectroscopic analysis.

The subfraction H gave several spots on TLC which was rechromatographed over CC eluted with n-hexane: EtOAc (1:10-10:1) to obtain three subfractions H01- H03. The subfraction H03 was rechromatographed over CC using n-hexane: EtOAc (2:3-1:4) to afford compound **4**.

3.3.1 3-Formyl-6,7-dimethoxy-furo[1,2]carbazole (**1**):

Yellow crystall; mp: 195-196; UV (MeOH) Λ_{max} nm (log ε): 204 (4.10), 234(4.37), 245 (4.22), 278 (4.34), 285 (4.21), 332 (3.96) and 361 (3.90); IR (KBr) ν_{max} cm^{-1}: 965, 1190, 1510, 1578, 1631, 1658, 3263 and 3400; ^{1}H NMR (400MHz,) δ = 10.09 (1H, s, HCO), 8.54 (1H, s, H-4), 7.83 (1H, s, H-2'), 7.59 (1H, s, H-5), 7.36 (1H, s, H-1'), 7.02 (1H, s, H-8), 3.82 (3H, s, 7-OCH_3), 3.78 (3H, s, 6-OCH_3); ^{13}C NMR (100MHz,) δ = 187.5 (CHO), 155.2 (C-5), 147.3 (C-2), 144.2 (C-7), 142.9 (C-6), 138.6 (C-2'), 138.2 (C-9a), 120.1 (C-8a), 120.0 (C-4b), 118.6 (C-4a), 114.8 (C-3), 111.7 (C-1), 103.6 (C-5), 102.8 (C-1'), 92.6 (C-8), 56.3 (OCH_3), 56.0 (OCH_3); ESI–TOF–MS: (m/z) 295.17 $[M+H]^+$ (calcd. for $C_{17}H_{13}NO_4$, 295.08). EI-MS m/z; 267 (M-CO), 265 (M-OCH_3) and 235 (M-2OCH_3).

3.3.2 Methyl-6,7-dimethoxy-furo[1,2]carbazole-3-carboxylate (**2**):

Yellow needles; mp 203 - 204; UV (EtOH) λ_{max} nm (log ε): 230 (4.20), 245 (4.08), 285 (4.35), 296 (4.25), 349 (3.80). IR (KBr) ν_{max} cm^{-1}: 3440, 1705, 1615, 1580, 1227, 1195, 1040, 815; ^{1}H NMR (400MHz) δ = 8.66 (1H, s, H-4), 7.85 (1H, d, J = 7.6 Hz, H-2'), 7.56 (1H, s, H-5), 7.29 (1H, d, J = 7.6 Hz, H-1'), 7.04 (1H, s, H-8), 3.92 (3H, s, CH_3OCO), 3.75 (3H, s, 6-OCH_3), 3.80 (3H, s, 7-OCH_3); ^{13}C NMR (100MHz) δ = 165.4 (CO), 154.1 (C-2), 147.3 (C-7), 144.4 (C-6), 142.4 (C-2'), 138.7 (C-9a), 137.5 (C-8a), 121.1 (C-4a), 120.6 (C-4b), 118.9 (C-4), 114.5 (C-3), 113.0 (C-1), 104.1 (C-1'), 103.3 (C-5), 92.8 (C-8), 56.3 (7-CH_3O), 56.0 (6-CH_3O), 52.4 (CH_3CO); ESI–TOF–MS: (m/z) 325.21 $[M+H]^+$ (calcd. for $C_{22}H_{25}NO_5$, 325.10). EI-MS m/z; 295 (M-OCH_3) and 265 (M-$COOCH_3$).

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