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Original Article

1-D and 2D-NMR Assignments of Nigricin from *Iris imbricata*

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Abstract

Ethanolic crude extract of *I. imbricata* Lindl. (Iridaceae) was subjected to column chromatography on silica gel with varying portions of MeOH: $CHCl_3$. Nigicin (irisolone) was isolated and its identification carried out by IR, UV, MS, 1-D and 2-D NMR spectroscopy.

Keywords: *Iris imbricata*; nigricin; isoflavonoid.

Introduction

Iris genus (Iridaceae family) is a rich source of isoflavoniods. Among different type of isoflavonoids: isoflavones, coumaranochromens, rotenoids and peltogynoids are present in this genus (1-3). Isoflavones have a wide range of biological activities such as antioxidant, cancer chemopreventive, anti-microbial, anti-inflammatory and phytoesterogenic properties (4-6). Twenty species of Iris grow in Iran (7-8). Our previous phytochemical study led to isolation and determination of two isoflavones from *I. songarica* (9). *I. imbricata* Lindl. is a native Iranian species which has not been studied so far (7-8). In this article ^{1}H-NMR and 2D-NMR assignments of nigricin are reported from this species.

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Experimental

General experimental procedure

The UV spectra were obtained using a Hitachi U-3200 spectrophotometer. The FT-IR spectra were recorded on a Vector 22 instrument. The ^{1}H-NMR was recorded on a Bruker AMX 500 NMR (Avance) instruments using the UNIX data system at 500 MHz using DMSO-d_6 as solvent. ^{1}H-^{13}C HMBC and HMQC were recorded at 500 MHZ (proton) and 125 MHZ (carbon), respectively. EI MS spectra were recorded on a Finnigan MAT 312. FAB mass measurements were performed on Jeol JMS HX 110 mass spectrometer using glycerol as the matrix. HREI MS was carried out on Jeol JMS 600 mass spectrometer. Column chromatography was carried out on silica gel (M&N), 70-230 mesh. Compounds on the TLC (M&N) were detected at 254 and 366 nm and by ceric sulfate as spraying reagent.

Plant material

The under ground parts of *I. imbricata* were collected near Siahbisheh in altitude 2500 m, Mazandaran Province, Iran, in June 2002 and identified by Ms. N. Mazhari (National Iranian Botanical Garden, Tehran, Iran). A voucher specimen (no.4078) was deposited at the Herbarium of agriculture faculty, Islamic Azad University, Karaj, Iran.

Extraction and isolation

The fresh rhizomes and roots of *I. imbricata* were cleaned under running tap water, cut into pieces and dried under shadow for two weeks. The powdered crude materials (1.2 Kg) were extracted by maceration with EtOH (80%) (3×8 lit.). The extract was evaporated and freeze dried to give 238 g. of gummy extract. The extract (20g) was chromatographed on silica gel column (70-230 mesh, 300 g) and eluted with varying portions of $CHCl_3$ and MeOH. Nigricin was purified from eluent $CHCl_3$:MeOH (98:2) and found to be the major isoflavone of the plant.

Result and disscution

Ethanolic extract of root and rhizome of *I. imbricata* was subjected to column chromatography on silical gel and eluted with varying portions of $CHCl_3$: MeOH (100:0- 96:4). Nigricin was purified from eluent $CHCl_3$: MeOH (98:2) and found to be the major isoflavone of this plant. The structure was elucidated using ^{1}H-NMR, HMBS, HMQC, EI MS, HR MS, IR and UV techniques.

The ^{1}H-NMR spectrum (Figure-1) of the compound showed the presence of five aromatic protons, a methylenedioxy group and a methoxyl group along with a hydroxyl group (4). A singlet at δ= 9.53 ppm indicated a hydroxyl group (C-4′) attached to B-ring which was further confirmed by COSY, HMQC and EI MS (10-11). A singlet at δ = 8.17 ppm was assigned to H-2 which further established by HMQC δ_H/δ_C: 8.2/154 (11-12) and showed the compound would be isoflavone (10-11). Two peaks at 7.31ppm (d, *J*= 8.44 Hz, 2H) and 6.77(d, *J*= 8.44 Hz, 2H) showed the presence of four aromatic proton attached to C-2′, 6′ and C-3′, 5′respectively which were reconfirmed by COSY and HMBC (10). A sharp singlet at δ= 6.15ppm (2H) as well as a cross peak δ_H/δ_C: 6.15/102, appeared in HMQC spectra revealed the presence of methylenedioxy group (10-11). On the other hand, HMQC showed that singlet at δ = 6.97ppm was coupled with C-8 (δ_H = 6.5-6.9 & δ_C = 90-96) which confirmed the position of methylenedioxy at C_6-C_7 but not at C_7-C_8.(11). A methoxy group appeared as a singlet at δ = 3.87ppm. HMBC experiment showed that this methoxy substituent is located at C-5 (4, 12). The EI MS spectrum showed a molecular ion at m/z 312 which was confirmed to be molecular weight by FAB^+ and FAB^-. The fragments at m/z 194 and 118 resulted from retro Diels-Alder fragmentation. The result indicated that compound has one methlenedioxy group along with one methoxy group attached to A-ring (4, 9, 11). The position of hydroxyl group on B-ring was by the ^{1}H-NMR. The splitting pattern of four aromatic protons on the B-ring was charachteristic of para disubstitution. This was further confirmed by HMBC and COSY. The UV spectrum exhibited absorption maxima at 264 and 322 nm which are characteristic for a trioxygenated A-ring of an isoflavone derivative (10-11). The UV spectrum by the application of chemical shift reagents ($AlCl_3$, HCl) showed absorbance at λ_{Max} 264 and 322_{nm} (MeOH) without any shift indicating the absence of a free radical hydroxyl group at C-5 and substitution at C-3′and 5′(10-11). IR absorption bands were found at 3100 (free OH), 1635 (C=O) and 922 (OCH_2O). The compound is determined to be nigricin (Irisolone) by means of ^{1}H-NMR, HMBC, HMQC, COSY, EI MS, HR MS, IR and UV techniques. Nigricin has been reported from *I. germanica, I. florentina, I. nigricans* and *I. nepalensis* previously (5, 11, 13, 14). In this paper we are reporting the full 2-D assignment of nigricin. For the first time compounds which were reported from *I. songarica* had 2′-hydroxylation pattern (9) while nigricin had a 4′-hydroxyl substituent. Nigricin has shown a very strong anti-inflammatory activity based on the reduction of highly water-soluble tetrazolium salt WST-1 in the presence of activated neutrophils (5). However no lipoxygenase inhibitory activity has been found for this compound according to

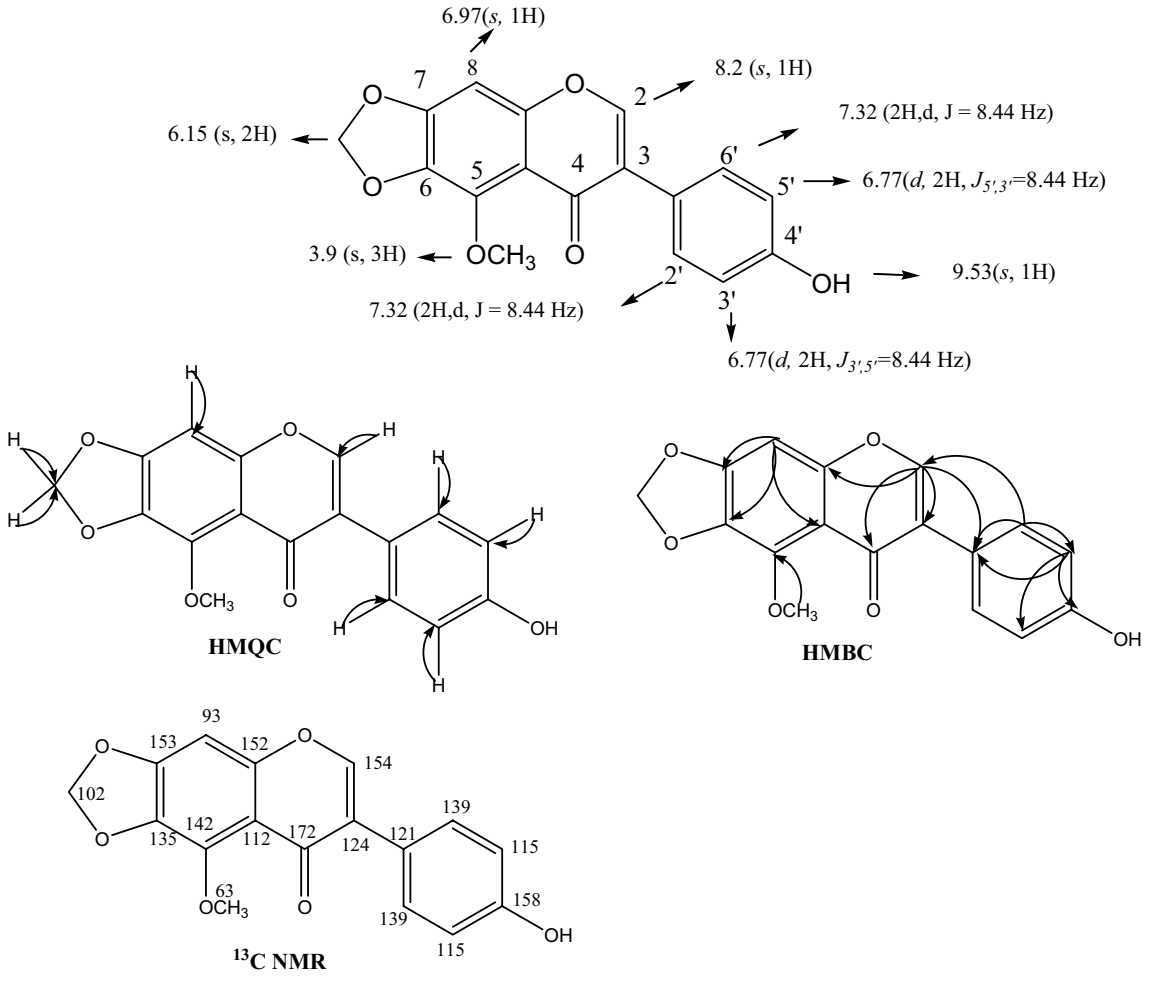

Figure 1. 1 & 2-D assignments of nigricin.

Nigricin A: White amorphous powder: **HR MS** $[M]^+$ at m/z 312.06811 (calcd. For $C_{17}H_{14}O_6$, 312.06336). **FAB MS** $[M+1]^+$: 313. **FAB MS** $[M-1]^+$: 311. **EI MS** m/z (rel.int): 312(54), 284(100), 266(79.23), 166(35.96), 118(62.74).**UV** λ_{Max} (MeOH): 264.2,322; +$AlCl_3$ without shift. **IR** *v*Max. (KBr) cm^{-1}: 3100 (OH free), 1635.5 (C=O), 1602.7, 1473.5, 1257, 921.9 (OCH_2O).**^{1}H-NMR** (DMSO-d6): δ 3.9 (*s*, 3H, OCH_3), 6.15 (2H, *s*, OCH2O), 6.77(2H, d, J= 8.44 Hz, $H_{3',5'}$), 6.97(1H, *s*, H_8), 7.32(2H, *d*, J= 8.44 Hz, $H_{2',6'}$), 8.2(1H, *s,* H_2), 9.53(1H, *s*, 4'-OH)

the method used by Riaz et al. (15). Therefore it seems that the anti-inflammatory activity of nigricin is mediated by a mechanism different from lipoxygenase inhibition and this mechanism could be a subject for further investigation.

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