Turk J Chem
24 (2000) , 191 – 197.

Flavonol Glycosides from *Consolida armeniaca*

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Received 03.08.1998

Three known flavonol glycosides, i.e., kaempferol 3,7-O-α-L-dirhamnopyranoside (**1**), kaempferol 3-O-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside-7-O-α-L-rhamnopyranoside (**2**) and kaempferol 3-O-α-L-rhamnopyranoside-7-O-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside (**3**), were isolated for the first time from the flowers of *Consolida armeniaca*, and the structures of flavonol glycosides were deduced by high field 1D and 2D NMR and FAB-MS spectroscopies. The acetyl derivatives of **1-3** were also prepared (**1a, 2a, 3a**) and identified by NMR and positive FAB mass spectra.

Key Words: *Consolida armeniaca,* flavonol glycosides, acetylated flavonol compounds.

Introduction

Consolida armeniaca is a plant widespread in Turkey[1] where it is used in folk medicine. In an investigation of a methanolic extract of *C. armeniaca*, three flavonol glycosides (**1,2,3**)[2−5] were isolated for the first time from this plant. Flavonol glycosides are common natural compounds which have been isolated from a wide variety of plants[2−12]. The present paper describes the isolation and identification of **1-3** through spectral analyses.

Experimental

Instruments: NMR spectra were recorded on a Bruker 400 MHz instrument in DMSO-d_6 and $CDCl_3$ using TMS as internal standard, and NOESY experiments were recorded on a Varian Gemini 200 MHz using a Varian NOE diff. program. (+) FAB-MS spectra were recorded on a VG-Zapspec MS instrument using NOBA matrix. Flash column chromatography was performed on a Si gel 60 G and reverse-phase C_{18} Silica and preparative TLC was performed with precoated Si gel F_{254} (20×20 cm 0.5 mm) plates. A

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voucher specimen has been deposited in the Herbarium of the Department of Biology at Karadeniz Technical University (KTUBH).

Extraction and Isolation of Flavonoids: Flowers of *Consolida armeniaca* were collected in the Gümüşhane-Bayburt region, in the north of Turkey, in July 1995. The identification of this species was made according to the Flora of Turkey (Davis, 1965)[1]. Air-dried flowers were blended in a Waring Blender. Blended flowers were placed in a Soxhlet extractor and extracted with MeOH for 12 h. MeOH solution was evaporated in a rotary evaporator in vacuo to dryness at 30-35°C and was dissolved in water and extracted with petroleum ether (40-60°), chloroform and ethyl acetate, successively. Ethyl acetate extract was evaporated in a rotary evaporator in vacuo to dryness at 30-35°C. The obtained crude mixture was chromatographed by Si gel 60 G (60 g, 230-400 mesh) column chromatography. Following elution with ethyl acetate, 60 fractions were collected (*ca.* 15-20 ml each). After analysis by TLC, fractions 1-25 were combined containing compounds **1** and **2**, and fractions 26-60 were combined containing compounds **2** and **3**. The first collected fraction was rechromatographed by C_{18} reversed-phase column chromatography. The column was eluted with discontinuous gradient of water-acetone (80:20 - 70:30) to give 32 fractions (*ca.* 1-3 ml each). After the reverse-phase TLC analysis, fractions 4-9 and 21-27 were combined. Each combined fraction was purified by Si gel (20×20, 0.5mm) preparative TLC using EtOAc-MeOH-water (100:13:10) solvent system to give compounds **1** (R_f = 0.59, 42 mg) and **2** (R_f = 0.40, 21 mg).

The second collected fraction was again chromatographed by C_{18} reversed phase column chromatography. The column was eluted with discontinuous gradient of water-acetone (90:10 - 60:40) to give 17 fractions (*ca.* 2-4 ml each). After the reverse-phase TLC analysis, fractions 2-8 and 11-17 were combined. Each combined fraction was purified by Si gel (20x20, 0.5mm) preparative TLC using EtOAc-MeOH-water (100:13:10) to give compounds **2** (R_f = 0.40, 12 mg) and **3** (R_f = 0.25, 35 mg). Compounds **1, 2** and **3** were checked by TLC Si gel plates using the following eluents: EtOAc-MeOH-Water (100:15:10) (eluent A), EtOAc-AcOH-HCOOH-Water (100:11:11:27) (eluent B), MEK(methyl ethyl ketone)-EtOAc-HCOOH-Water-Benzene (4:3:1:1:2) (eluent C).

Acid Hydrolysis of 1, 2 and 3: A solution of each compound (5 mg) in aqueous 2M HCl (5 ml) was heated in a water bath at 100° for 2 h. The mixture was evaporated in a rotary evaporator in vacuo under N_2 to dryness and was solved in MeOH. The aglycone was detected on Si gel plates under UV_{366} and with NH_3 vapour. Sugar moieties were detected on cellulose TLC plates with EtOAc-MeOH-Water-AcOH (13:3:3:4) using aniline phatalate as spraying reagent.

Acetylation of 1, 2, 3 (1a, 2a, 3a): Each compound (25 mg) was solved in pyridine-acetic anhydride (1:1, 2 ml) and heated in water bath at 80° for 4 h. The mixture was evaporated at rotary evaporator in vacuo under N_2 to dryness. Each peracetylated compound was purified by Si gel preparative TLC using $CHCl_3$-MeOH (15:3) to give compounds **1a** (R_f = 0.51, 29 mg), **2a** (R_f = 0.45, 35 mg), **3a** (R_f = 0.40, 31 mg).

Kaempferol-3-O-α-L-rhamnoside-7-O-α-L-rhamnopyranoside (1): TLC R_f : $0.59^{(A)}$, $0.75^{(B)}$, $0.55^{(C)}$; UV $\lambda_{\max}^{MeOH}$ nm: 203, 265, 343; +NaOMe: 209, 247, 270, 391; +NaOAc: 222, 264, 364; +$AlCl_3$: 203, 274, 301, 350, 400; + $AlCl_3$+HCl: 203, 274, 345, 397. Positive FAB-MS (matrix, NOBA) *m/z* 579 $[M+1]^+$, 431 $[M\text{-Rha}]^+$, 286 $[\text{aglycone}]^+$. ^1H and ^{13}C NMR data, see Table 1.

Kaempferol-3-O-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside-7-O-α-L-rhamnopyranoside (2): TLC R_f: $0.25^{(A)}$, $0.30^{(B)}$, $0.083^{(c)}$; λ_{max}^{MeOH} nm: 204, 265, 339; +NaOMe: 210,

272, 393; +NaOAc: 225, 263, 382; +$AlCl_3$: 205, 274, 348, 396; +$AlCl_3$+HCl: 205, 274, 346, 396. Positive FAB-MS (matrix, NOBA) *m/z*; 577 [M-Glc]$^+$ 431 [M-Rha-Glc]$^+$, 287 [aglycone]$^+$. ^{1}H and ^{13}C NMR data, see Table 1.

Table 1. ^{1}H and ^{13}C NMR spectral data of the flavonoid compounds 1-3 in DMSO-d_6.

	1[a,b,c]		**2**[a,b,c]		**3**[a,b,c]	
C No	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR
2	157.5	-	158.7	-	158.1	-
3	134.4	-	134.0	-	135.6	-
4	178.4	-	177.0	-	177.5	-
5	161.9	-	161.0	-	161.0	-
6	100.8	6.35 br s	101.4	6.18 br s	101.9	6.36 br s
7	162.5	-	162.4	-	162.5	-
8	95.2	6.67 br s	[d]	6.45 br s	95.2	6.64 br s
9	156.1	-	154.8	-	[d]	-
10	107.2	-	105.1	-	105.3	-
1'	121.3	-	121.2	-	121.4	-
2', 6'	131.4	7.78 d, *J*=8.1 Hz	131.4	7.72 d, *J*= 8.3 Hz	131.4	7.79 d, *J*= 8.3 Hz
3', 5'	116.5	6.93 d, *J*=8.1 Hz	116.9	6.82 d, *J*= 8.5 Hz	116.5	6.93 d, *J*= 8.5 Hz
4'	161.4	-	159.2	-	161.3	-
3-O-Rh 1"	100.7	5.32 br s	101.4	5.55 br s	100.7	5.55 br s
2"	70.9	3.86	81.9	4.13	70.9	3.38
3"	71.2	3.62	71.2	3.58	71.0	3.58
4"	72.8	3.55	72.5	3.18	71.5	3.18
5"	70.7	3.18	71.1	3.40	71.3	3.40
6"	18.9	0.82 d, *J*= 6.3 Hz	18.3	0.86 d, *J*= 5.3 Hz	18.3	0.88 d, *J*= 5.8 Hz
Glc 1""			106.7	4.28 d, *J*=7.5 Hz	106.7	4.28 d, *J*=7.5 Hz
2""			74.8	3.39	74.8	3.39
3""			77.4	3.14	77.4	3.16
4""			70.8	3.60	70.8	3.58
5""			77.2	3.32	77.1	3.34
6""			61.7	3.38	61.5	3.39
7-O-Rh 1'"	99.2	5.55 br s	99.0	5.46 br s	99.2	5.53 br s
2'"	70.6	3.87	70.8	3.38	82.0	4.12
3'"	71.3	3.65	71.2	3.32	71.2	3.42
4'"	71.9	3.32	72.5	3.67	72.5	3.37
5'"	70.4	3.45	70.5	3.30	71.2	3.30
6'"	18.4	1.15 d, *J*= 6.1Hz	18.8	1.13 d, *J*= 6.1 Hz	18.4	1.13 d, *J*= 5.9 Hz

[a] Chemical shifts (ppm) are relative to internal TMS.
[b] Assignments based on DEPT, 2D-COSY and HETCOR spectra.
[c] Quaternary carbons were assigned with comparison of literature values2-17.
[d] ^{13}C NMR peak not observed.

Kaempferol-3-O-α-L-rhamnopyranoside-7-O-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside (3): TLC R_f: $0.40^{(A)}$, $0.57^{(B)}$, $0.22^{(C)}$; UV λ_{max}^{MeOH} nm: 203, 267, 330; +NaOMe: 210, 270, 371; +NaOAc: 226, 266, 321; +$AlCl_3$: 205, 276, 302, 320, 396; +$AlCl_3$+HCl: 203, 277, 301, 323, 396. Positive FAB-MS (matrix, NOBA) *m/z*; 577 [M-Glc]$^+$, 431 [M-Rha-Glc]$^+$, 287 [aglycone]$^+$. ^{1}H and ^{13}C NMR data, see Table 1.

Kaempferol-3-O-α-L-rhamnoside-7-O-α-L- rhamnopyranoside octa acetate (1a): Positive FAB-MS (matrix, NOBA) *m/z* 915 [M]$^+$, 642 [M-Rha]$^+$, 370 [aglycone]$^+$. ^{1}H and ^{13}C NMR data, see Table 2.

Kaempferol-3-O-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside-7-O-α-L-rhamnopyranoside deca acetate (2a): Positive FAB-MS (matrix, NOBA) *m/z* 1203 [M]$^+$, 872 [M-Glc]$^+$, 930 [M-Rha]$^+$, 643 [M-Rha-Glc]$^+$, 370 [aglycone]$^+$. ^{1}H and ^{13}C NMR data, see Table 2.

Kaempferol-3-O-α-L-rhamnopyranoside-7-O-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside deca acetate (3a): Positive FAB-MS (matrix, NOBA) *m/z* 1203 [M]$^+$, 872 [M-Glc]$^+$, 930 [M-Rha]$^+$, 643 [M-Rha-Glc]$^+$, 370 [aglycone]$^+$. ^{1}H and ^{13}C NMR data, see Table 2.

Table 2. ^{1}H and ^{13}C NMR spectral data of the acetylated flavonoid compounds **1a, 2a,** and **3a** in $CDCl_3$.

	1a[a,b,c]		**2a**[a,b,c]		**3a**[a,b,c]	
C No	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR
2	156.1	-	155.2	-	155.2	-
3	137.1	-	137.5	-	137.5	-
4	174.2	-	173.0	-	173.0	-
5	160.1	-	160.2	-	160.2	-
6	109.8	6.65 br s	109.8	6.78 br s	109.8	6.74 br s
7	159.5	-	159.1	-	159.1	-
8	102.3	7.10 br s	102.3	7.10 br s	102.3	7.10 br s
9	153.2	-	153.1	-	153.1	-
10	112.1	-	112.6	-	112.6	-
1'	126.3	-	127.8	-	127.8	-
2', 6'	130.5	7.90 d, *J*=8.1 Hz	130.6	7.88 d, *J*=8.3 Hz	130.6	7.90 d, *J*=8.3 Hz
3', 5'	122.5	7.28 d, *J*=8.1 Hz	122.4	7.30 d, *J*=8.5 Hz	122.4	7.28 d, *J*=8.5 Hz
4'	151.6	-	151.7	-	151.7	-
3-O-Rh 1"	99.7	5.58 br s	100.7	5.58 br s	100.7	5.58 br s
2"	69.6	5.48	78.0	4.43	69.5	5.45
3"	69.3	5.32	73.0	5.18	68.9	5.43
4"	70.9	4.96	71.1	4.90	68.7	5.22
5"	68.8	3.48	68.6	3.38	68.4	3.95
6"	17.4	0.94 d, *J*=6.1 Hz	17.4	0.88 d, *J*=6.3Hz	17.8	1.21 d, *J*=6.1Hz
Glc 1""			102.7	4.60 d, *J*=7.5Hz	102.7	4.58 d, *J*=7.5Hz
2""			71.5	5.05	71.5	5.05
3""			70.8	5.20	70.8	5.20
4""			70.9	5.12	70.9	5.13
5""			72.1	3.72	72.1	3.70
6""			62.0	4.28, 4.05	62.0	4.28, 4.05
7-O-Rh 1"'	96.2	5.60 br s	96.3	5.59 br s	96.2	5.58 br s
2"'	69.5	5.68	69.5	5.43	78.0	4.42
3"'	69.0	5.47	68.9	5.42	73.0	5.14
4"'	70.8	5.20	68.7	5.18	71.1	4.91
5"'	68.4	3.92	68.4	3.90	68.6	3.40
6"'	17.8	1.24 d, *J*=6.2 Hz	17.8	1.23 d, *J*=6.1Hz	17.4	0.94 d, *J*=6.2 Hz

[a] Chemical shifts (ppm) are relative to internal TMS.
[b] Assignments based on DEPT, 2D-COSY, HETCOR and NOESY spectra.
[c] Quaternary carbons were assigned with comparison of literature values.
Additional proton signals for **1a**: δ 2.31, 2.41 (arom. Ac x 2), δ 1.91-2.18 (aliph. Ac x 6); **2a**: δ 2.31, 2.41 (arom. Ac x 2), δ 1.94-2.18 (aliph. Ac x 9); **3a**: δ 2.32, 2.41 (arom. Ac x 2), δ 1.94-2.20 (aliph. Ac x 9).
Additional carbon signals for **1a**: δ 169-173 (C=O x 8); **2a**: δ 170-172 (C=O x 11); **3a**: δ170-173 (C=O x 11).

Results and Discussion

The flowers of *Consolida armeniaca* were air-dried and exhaustively extracted with method. The methanol residue, diluted with water, was sequentially extracted with petroleum ether, chloroform and ethyl acetate. From these extracts, three flavonol glycosides were isolated. Compounds **1-3** were sugar substituted at C-3 and C-7 with free hydroxyl groups at C-5 and C-4′ as indicated by their UV spectra (see Experimental) with addition of diagnostic shift reagents[6−7]. Anomeric configurations, linkage sites and sequence of sugars in the flavonol glycosides can be determined using various NMR experiments, ^{1}H NMR chemical shifts and vicinal coupling constants, ^{13}C NMR chemical shifts and ^{13}C-^{1}H coupling constants, interresidue NOE and long-range homo- and heteronuclearcorrelations. Usually the anomeric resonances of α-glycosides resonate at a

down-field position by 0.3-0.5 ppm compared with that of the corresponding β-glycosides. Thus, resonances at the lowest field (4.5-5.5 ppm), which are doublets with $^3J_{1,2}$ in the range 1-4 Hz, are of α-anomeric protons, whereas β-anomeric protons appear as doublets between 4.0 and 4.8 ppm with $^3J_{1,2}$ in the range 6-8 Hz in monosaccharides stereochemistry.

From consideration of the ^{1}H, ^{13}C and DEPT NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY spectral data, compounds **1-3** were shown to be kaempferol 3,7-derivatives containing two rhamnoses (**1**) and two rhamnoses and one glucose (**2** and **3**). This is the first report on the occurrence of di- and tri-glycosylated flavonols in this plant.

The ^{1}H NMR spectrum of compound **1** (Table 1) showed the expected signals of aromatic protons at δ 7.78 (d, J=8.12 Hz) for H-2′, H-6′ and δ 6.93 (d, J=8.12 Hz) for H-3′, H-5′ and broad singlet signals at δ 6.67 and 6.35 were assigned to H-6 and H-8, respectively. In the NMR spectrum two doublets, at δ 0.82 (d, J=6.3 Hz) and 1.15 (d, J=6.1 Hz), indicated the presence of two sets of rhamnose methyl protons and the signals for the anomeric protons appeared at δ 5.55 (br s) and 5.32 (br s) ppm, respectively. The signals for the carbon atoms of two rhamnose moieties were also observable in the ^{13}C NMR spectrum (Table 1). Comparison of ^{1}H and ^{13}C NMR spectra of **1** confirmed the presence of two rhamnose moieties in the molecule[3,7,10]. After the acid hydrolysis of **1**, the presence of rhamnose was detected by TLC.

In the ^{13}C NMR spectrum of **1** (Table 1), the presence of signals at δ 134.4 and 162.5 agreed with the glycosylation at C-3 and C-7 positions[6−9]. Two anomeric carbon signals were observed at δ 99.2 and 100.7. The remaining sugar carbons appearing at δ 18.4 -72.8 were characterised from NMR data and showed the sugars to be in pyranose form[3,7,10].

The positive FAB mass spectrum of **1** exhibited a molecular ion at *m/z* 579 $[M+H]^+$, and an aglycone ion peak at 286 $[kaempferol+H]^+$ and another ion peak at 431 $[M\text{-}Rha+H]^+$. From the above spectral data compound **1** is therefore kaempferol 3,7-di-O- α-L-rhamnoside.

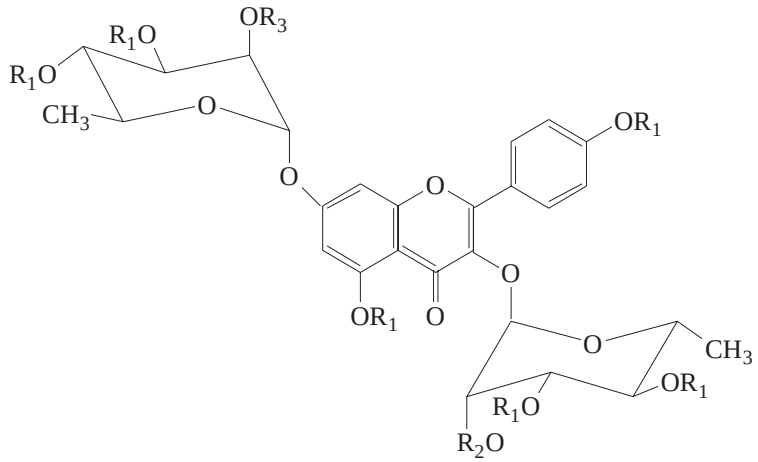
The ^{1}H-NMR and UV spectral data of the aglycone of **2** (see Experimental) indicated an identical pattern to **1**. A comparison of the ^{1}H and ^{13}C NMR spectra of **2** with that of **1**, showed that the signals for H-2, H-3′, H-5′, H-6′, H-6 and H-8, and C-2, C-10, C-1′ and C-6′ (Table 1) were similar. Thus, the aglycone of **2** is also kaempferol. The ^{1}H NMR spectrum of **2** (Table 1) contained three anomeric proton signals at δ 4.28 (d, J=7.5 Hz), 5.46 (br s) and 5.55 (br s) attributable to C-3,7-disubstitution of kaempferol[6−9].

The presence of three sugars in **2** was apparent from the three anomeric carbon signals (δ 99.0, 101.4, 106.7) as well. Two of these sugars were α-L-rhamnoses (δ 99.0 and 101.4) suggested by the distinct anomeric protons at δ 5.46 (br s), 5.55 (br s) and methyl protons at δ 0.86 and 1.13 ppm [7]. The third sugar was identified as β-D-glucose by the distinct anomeric proton at δ 4.28 (d, J=7.5 Hz) correlating with the carbon signal at β 106.7 [3,7−10]. The sequence and the position of the sugars were determined from the NMR and FAB mass spectra[3,7−12]. From the fragmentation pattern and NMR spectra, rhamnoses were attached directly to C-3 and C-7 positions of compound **2**. The signal for C-2″ of rhamnose was at δ 81.9. We observed a downfield shift relative to **1** due to the interglycosidic bond between C-2″ and C-1‴′. Therefore, interglycosidic linkage was found to be from glucose (1→2) rhamnose position in 2[3,7−8]. The positive FAB mass spectrum of compound **2** did not show a molecular ion peak, but other typical fragments due to the loss of glucose at *m/z* 577 $[M\text{-}Glc]^+$ and $[M\text{-}Rha\text{-}Glc]^+$ at *m/z* 431 and at 287 $[kaempferol]^+$ were seen in the FAB mass spectrum. On the basis of these spectral data, **2** was identified as kaempferol

3-O-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside-7-O-α-L-rhamnopyranoside from *C. armeniaca*.

The third compound showed a pseudomolecular ion peak at *m/z* 579 $[M+H]^+$ in the positive ion FAB mass spectrum. The UV spectrum of **3** showed the characteristic bands of a kaempferol, like compounds **1** and **2** [2−3,7], substituted at positions C-3 and C-7 [6−8]. The ^1H and ^{13}C NMR spectra confirmed this hypothesis (Table 1). From NMR and FAB-MS spectral analysis, compound **3** is kaempferol triglycoside. Three sugar moieties were identified as two rhamnose and one glucose from the ^{13}C NMR data [3,7−12] and TLC after the acid hydrolysis [3,7−10]. Analysis of the NMR spectral data and positive FAB-MS fragmentation of compound **3** indicated that rhamnose units were directly attached to C-3 and C-7 positions. The interglycosidic nature of glucose was clearly observed from the chemical shifts of H-2‴ and C-2‴ in the NMR spectra (Table 1). Thus, on the basis of these data, compound **3** is kaempferol 3-O-α-L-rhamnopyranoside-7-O-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside.

The nature of the sugar moieties of compounds **1-3** could be entirely established on the basis of NMR data. Particular attention was paid to the very crowded mid-field region of the ^1H NMR spectrum, characterised by many overlapping signals. In order to overcome this difficulty, compounds **1-3** were acetylated to improve the signal resolution, thus obtaining the octa acetate of **1**, **1a** and the deca acetate of **2** and **3, 2a** and **3a** (see Experimental). Using 1D ^1H, ^{13}C and DEPT NMR, 2D ^1H-^1H COSY, ^1H-^{13}C-COSY and NOESY spectra, acetylated compounds **1a, 2a**, and **3a** were identified (Table 2). The FAB-MS of compounds **1a, 2a**, and **3a** showed pseudomolecular ion peaks at *m/z* 915 $[M]^+$, **1a**, 1203 $[M+H]^+$, **2a** and 1203 $[M+H]^+$, 3a, and other fragments[7−10]. Acetylated compounds **1a, 2a**, and **3a**, confirmed the structures of **1-3** as assigned form.



	R_1	R_2	R_3
1	H	H	H
1a	Ac	Ac	Ac
2	H	Glc	H
2a	Ac	Ac-Glc	Ac
3	H	H	Glc
3a	Ac	Ac	AcGlc

Acknowledgments

This work was supported by the Karadeniz Technical University Research Foundation. Thanks to Dr. İnci Şahin and Prof. Dr. Tuncel Özden for recording the 400 MHz NMR spectra. Thanks also TÜBİTAK-Gebze for recording the FAB-MS spectra and Mr. C. Kazaz for recording the NOESY spectra.

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