İstanbul Ecz. Fak. Mec.
33 (2000)

J. Fac. Pharm. Istanbul
33 (2000)

CHEMICAL CONSTITUENTS OF *CENTAUREA AMANICOLA*

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SUMMARY

The chemical investigation of an extract of the aerial parts of *Centaurea amanicola* afforded several compounds including flavon (apigenin), coumarin (scopoletin), triterpen (α-amyrin), steroid (β-sitosterol) and six aromatic compounds (vanilic acid, p-hydroxybenzoic acid, benzyl-β-D-glucoside, 2-phenylethyl β-D-glucoside, 4'-formyl-2',6'-dimethoxy-n-heptanophenone and 4'-formil-n-heptanophenone). This is the first report on the chemical constituents of *Centaurea amanicola*. The structures of the compounds were determined by spectroscopic methods such as UV, IR, ^{1}H and ^{13}C NMR spectroscopy as well as mass spectrometry.

ÖZET

Centaurea amanicola bitkisinin toprak üstü kısımları kimyasal olarak incelenmiş, çeşitli bileşikler flavon (apigenin), kumarin (scopoletin), triterpen (α-amyrin), steroid (β-sitosterol) ve altı adet aromatik bileşik (vanilic asit, p-hidroksibenzoik asit, benzil-β-D-glukosid, 2-peniletil β-D-glukosid, 4'-formil-2',6'-dimetoksi-n-heptanofenon ve 4'-formil-n-heptanofenon) elde edilmiştir. *Centaurea amanicola* türü ilk kez kimyasal

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bakımdan incelenmiştir. Bileşiklerin yapıları UV, IR, ^{1}H ve ^{13}C NMR spektroskopileri ve mass spektrometri yöntemlerinden yararlanılarak açıklanmıştır.

Key words: *Centaurea amanicola;* Compositae; flavon, coumarin; triterpene; steroid; aromatics.

INTRODUCTION

The large genus *Centaurea* from the family Compositae has been the subject of many phytochemical investigations which have shown sesquiterpene lactones (1), flavonoids (2) and acetylenes (3) to be common constituents. As a part of a systematic examination of *Centaurea* plants in Turkey, we have investigated *Centaurea amanicola* Hub-Mor. A survey of literature revealed no paper dealing with the chemical constituents of *C. amanicola*. Investigation of the aerial parts afforded benzyl-β-D-glucoside (**1**) (4), 2-phenyletil β-D-glucoside (**2**) (4), 4'-formyl-n-heptanofenon (**3**), 4'-formyl-2',6'-dimethoxy-n-heptanofenon (**4**), apigenin (**5**), scopoletin (**6**), α-amyrin (7), β-sitosterol (**8**), vanilic acid (**9**), p-hidroxybenzoic acid (**10**). To our knowledge compounds **3** and **4** are described for the first time in this paper.

RESULTS AND DISCUSSION

Compounds **1** and **2** were obtained as a mixture. Several attempts separation by preparative TLC were failed. They could not be separated by acetylation with pyridine/Ac_2O. However, the spectral properties allowed the assignment of the structures of **1** and **2**. In the ^{13}C NMR spectrum (see experimental), while the nonanomeric carbons of sugar moieties resonated in the same frequencies, the anomeric carbons could be observed separately at δ 102.0 (C-1) and 102.3 (C-1'). The aromatic carbons of both compounds were also assigned separately. The acetylated product of the mixture gave better resolved ^{1}H NMR spectrum in $CDCl_3$ in which anomeric protons (H-1 and H-1') were seen at δ 4.54 (δ, J= 7.5 Hz for **1**) and 4.48 (d, J= 7.8 Hz for **2**). Assignment of the CH_2 protons and the nonanomeric protons of the sugar part were achived by spin-decoupling experiments. On Irradiation of the doublet at δ 4.63 (J= 12.5 Hz, H-7a), the douplet at δ 4.91 (J= 12.5 Hz, H-7b) turned to a singlet indicating the presence of an isolated methylene group in compound **1**. On the other hand, irradiation of the triplet at δ 2.89 (J= 7 Hz, H-8') simplified the multiplet at δ 4.10 (H-7') and vice versa. This observation indicated that compound **2** had an additional CH_2 group. By irradiation of H-5 of the sugar part at *ca.* δ 3.60-3.70 (m), the sequences H-5 through H-2 were clearly

established. The only difference between **1** and **2** was in the number of CH_2 groups. While compound **1** had one CH_2 group, compound **2** had two CH_2 groups. The sugar part was deduced as glucose by acid hydrolysis. From the coupling constants of the anomeric protons (J =7.5 and 7.8 Hz for **1** and **2,** respectively) were indicative for the orientation of the glycosidic linkage to be β. On the basis of spectral data compounds **1** and **2** were identified as benzyl-β-D-glucoside and 2-phenyletil β-D-glucoside, respectively. These compounds are known natural constituents of various sources and isolation of as benzyl-β-D-glucoside and 2-phenyletil β-D-glucoside most frequently as mixtures has also been reported (4).

The IR spectrum of compound **3** showed strong absorptions of carbonyl stretching frequencies at 1710 cm^{-1} (HC=O) and 1690 cm^{-1} (C=O) and aromaticity at 1620-1480 cm^{1}. The ^1H NMR spectrum (see experimental) showed a p-substituted phenyl group resonances at δ 7.81 (2H, d, J=8.5 Hz, H-3'-H-5') and 6.95 (2H, d, J=8.5 Hz, H-2'-H-6'), an aldehyde proton at δ 9.87 (1H, s), a methyl group at δ 0.90 (3H, t, J= 6.8 Hz, H-7), and methylene groups signals at δ 2.36 (2H, t, J=7 Hz, H-2), 1.62 (2H, t, J=7 Hz, H-3) and at δ *ca* 1.30-1.20 (m, H-4, H-5, H-6). The assignments were made by spin decoupling experiments. Irridiaton of the multiplet at δ 1.30-1.20 turned the triplet of H-7 to a singlet and simplified the broad triplet of H-3 at δ 1.62. On the other hand, irradiation of the H-3 resonance (H_δ 1.62) collapsed the triplet of H-2 at δ 2.36 to a singlet indicating these two groups are linked to each other. The ^{13}C NMR (APT) spectrum exhibited 14 carbon atoms, including one CH_3, five CH_2, five CH and three quaternary carbons of which two are oxygenated (one aldehyde and one ketone). Based on the molecular ion at *m/z* 219 $[M+H]^+$ in the EI-mass spectrum and 6 degrees of unsaturation, of which four come from aromatic ring, two degrees of unsaturation were revealed for side chains. The signals at δ 190.7 (aldehyde) and 204.3 (ketone) confirmed this conclusion. The *n*-heptane side chain was deduced from the EI-mass spectrum (see experimental).

The spectral properties of compound **4** showed similarity to that of **3.** However, its ^1H and ^{13}C NMR spectra showed additional two methoxyl groups at δ 3.93 (6H, Cδ 56.5), and the two proton singlet at δ 7.16 (2H, H-3', H-5') in the ^1H NMR spectrum indicated symmetrical substitution on aromatic ring. The EI-mass spectrum of **4** established a molecular formula of $C_{16}H_{22}O_4$ with a molecular ion peak at *m/z* 279 $[M+H]^+$. All of the spectral data supported that compund **3** and **4** were 4'-formyl-n-heptanophenone and 4'-formyl-2',6'-dimethoxy-n-heptanophenone, respectively.

EXPERIMENTAL

General: ^{1}H (200 MHz) and ^{13}C (50.32 MHz) NMR spectra were recorded with a Brucker AC L instrument and chemical shifts are given on the δ (ppm) scale, TMS as an internal standard; IR spectra were measured with Perkin Elmer 1615 FT apparatus; MS were run with VG Zabspec GC-MS Instrument.

Plant Marterial: *Centaurea amanicola* Hub-Mor was collected from Amanos Mountain. A voucher specimen is deposited in the Herbarium of Faculty of Pharmacy, University of Istanbul (ISTE 49944).

Extraction and Isolation: Air-dried aerial parts (994 g) were cut into small pieces and extracted with petrolum ether-ether-ethanol (1:1:1) for 24 hr in room temp. The extract were distilled at 40 °C in *vacuo* to dryness. The crude extract (48 g) was subjected to Si gel column chromatography (250 g, Merck, Kieselgel 100, 70-230 Mesh ASTM, 80 x 3 cm) and eluted with petrol ether a gradient of ether up to 100%, and followed by EtOH (5%). Totally ten fractions were obtained. The similar fractions were combined and further separated on small Si gel columns when necessary. The single compounds were purified by prep. TLC chromatography.

Benzyl β-D-glucoside (1) and 2-phenylethyl β-D-glucoside (2): ^{1}H NMR ($CDCl_3$-CD_3OD, δ 200 MHz): 2.95, (t, *J*=7 Hz, H-8'), 4.10 (m, *J* =7 Hz, H-7'), 4.31 (1H, d, *J* =8 Hz, H-1'), 4.41 (1H, d, *J* =8 Hz, H-1), 4.66 (1H, d, *J* =12 Hz, H-7a), 4.91 (1H, d, *J* =12 Hz, H-7b), 7.20-7.40 (aromatic protons), 3.20-3.50 and 3.75-3.85 (nonanomeric protons of sugar parts). ^{13}C NMR ($CDCl_3$-CD_3OD, δ 50.32 MHz): 102.0 (C-1), 102.3 (C-1'), 73.5 (C-2, C-2'), 76.3 (C-3, C-3'), 69.4 (C-4, C-4'), 75.8 (C-5, C-5'), 63.5 (C-6, C-6'), 71.4 (C-7), 70.8 (C-7'), 137.1 (C-8), 36.1 (C-8'), 128.5 (C-9), 138.3 (C-9'), 128.2 (C-10-, C-11'), 128.9 (C-10'), 128.0 (C-11), 126.4 (C-12).

Benzyl-β-D-glucoside tetraacetate **(1a) and *2-Phenyletil β-D-glucoside tetraacetate*** **(2a)-** ^{1}H NMR ($CDCl_3$, δ, 200 MHz): 2.89 (2H, t, *J*= 7 Hz, H-8'), 3.60-3.70 (m, H-5, H-5'), 4.28 (dd, *J* =5, 12 Hz, H-6a), 4.16 (dd, *J* =2.5, 12 Hz, H-6b), 4.10 (m, H-7'), 4.48 (1H, d, *J* =7.8 Hz, H-1'), 4.54 (1H, d, *J* =7.6 Hz, H-1), 4.63 (1H, d, *J* =12 Hz, H-7a), 4.91 (1H, d, *J* =12.5 Hz, H-7b), 4.98 (1H, dd, *J*=9, 7.8 Hz, H-2'), 5.10 (1H, dd, *J* =9, 8 Hz, H-2), 5.18 (1H, t, *J* =9 Hz, H-3'), 5.11, (1H, t, *J* =9 Hz, H-3), 5.03 (1H, t, *J* =9 Hz, H-4') AcO (2.09, 2.07, 2.00, 1.98). ^{13}C NMR ($CDCl_3$, δ, 50.32 MHz): 102.0 (d, C-1), 102.3 (d, C-1'), 75.3 (d, C-2, C-2'), 76.3 (d, C-3, C-3'), 69.9 (d, C-4, C-4'), 75.8 (d, C-5, C-5'), 63.5 (t, C-6, C-6'), 71.4 (t, C-7), 70.8 (t, C-7'), 137.1 (s, C-8), 36.1 (t, C-8'), 128.5 (d, C-9), 128.2 (d, C-10), 128.9 (d, C-11), 139.6 (s, C-9'), 129.1 (d, C-10'), 128.0 (d, C-11'), 126.4 (d, C-12'), 170.0 (AcO), 169.8 (AcO).

Acetylation of 1 + 2: 6 mg of a mixture of **1** and **2** was refluxed with 1 ml of Ac_2O-pyridine (1:1) on a water-bath for 3 hr. The acetylated product gave a single large spot on Si gel TLC plates ($CHCl_3$-Me_2CO / 9:1) and could not be separated by prep. TLC.

Hydrolysis of 1 + 2: 5 mg of a mixture of **1** and **2** was treated with 2 ml of 2N HCl under reflux for 2 hr. The residue was poured in to small amount of water. Sugar part was identified as glucose on Si gel TLC plates (EtOAc-H_2O-MeOH-AcOH / 13:3:3:4) by comparison with standard sugar samples after treating with anilinphthalate reagent.

4'-Formyl-n-heptanofenon (3): UV λ_{max} (MeOH) nm: 285, 220; IR ν_{max} (KBr) cm^{-1}: 3200, 2920, 2840, 1710, 1690, 1620, 1480, 1185, 895, 720. ^1H NMR ($CDCl_3$ δ, 200 MHz): 0.89 (3H, t, J = 6.8 Hz, H-7), 1.62 (2H, t, J =7 Hz, H-3), 1.30 (2H, m, H-6), 2.36 (3H, t, J =7 Hz, H-2), 6.95 (2H, d, J = 8.5 Hz, H-2', H-6'), 7.81 (2H, d, J = 8.5 Hz, H-3', H-5'), 9.87 (1H, s, HC=O). ^{13}C NMR ($CDCl_3$, δ, 50.32 MHz): 201.0 (s, C-1), 190.7 (d, HC=O), 132.3 (d, C-3', C-5') 115.9 (d, C-2', C-6'), 33.4 (t, C-2), 31.9 (t, C-3), 29.6 (t, C-4), 24.7 (t, C-5), 22.4 (t, C-6), 14.1 (q, C-'7). EIMS *m/z* (rel. int.). 219 $[M+1]^+$ (6), 185 [219-CHO-CH_3] (16)), 170 [185-CH_2] (14), 156 [170-CH_2] (12), 142 [156-CH_2] (9), 138 [143-CH_2] (24), 124 [138-CH_2] (17), 85 [$C_7H_{13}O$] (43), 83 (64), 73 (90), 57 (100).

4'-Formyl-2',6'-dimethoxy-n-heptanofenon **(4)**- UV λ_{max} (MeOH) nm: 305, 220; IR ν_{max} ($CHCl_3$) cm^{-1}: 3040, 2920, 2840, 1700, 1670, 1600, 1510, 898, 720. ^1H NMR ($CDCl_3$, δ, 200 MHz): 0.90 (3H, t, J = 7 Hz, H-7), 1.62 (2H, t, J =7 Hz, H-3), 1.10-1.30 (6H, m, H-4, H-5, H-6), 3.93 (6H, s, OMe), 7.16 (2H, s, H-3', H-5'), 9.83 (1H, s, CHO). ^{13}C NMR ($CDCl_3$, δ, 50.32 MHz): 202.2 (s, C-1), 190.8 (d, HC=O), 106.7 (d, C-3', C-5'), 56.5 (OMe x 2), 24.6 (t, C-6), 29.6 (t, C-3, C-4, C-5), 34.9 (t, C-2), 14.7 (q, C-7). EIMS *m/z* (rel. int.). 279 $[M+1]^+$ (26), 182 (100), 166 [M^+-$C_7H_{13}O$] (27), 149 (41), 135 (166-OCH_3) (14), 113 $[C_7H_{13}O]^+$ (10), 85 (30), 69 (25), 57 (39).

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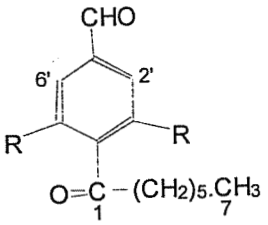

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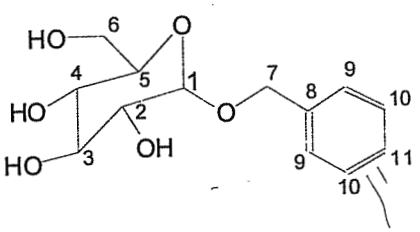
4 R = OCH3



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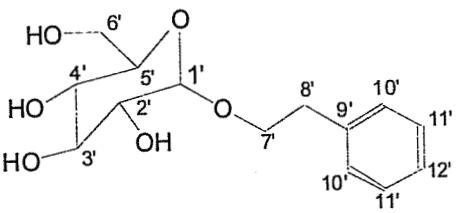

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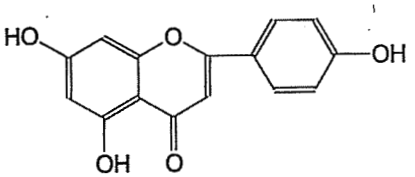

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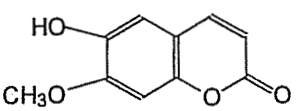

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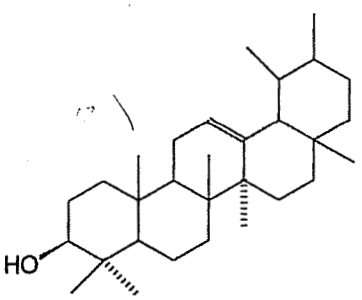

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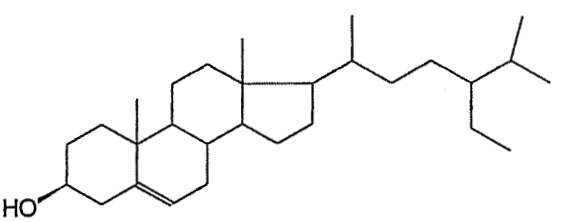

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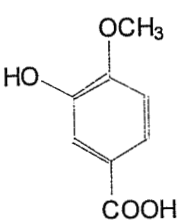

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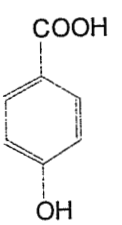

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