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Iridoid and Lignan Glucosides from *Bellardia trixago* (L.) All.*

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Abstract: Five iridoid glucosides aucubin (I), bartsioside (II), melampyroside (III), mussaenoside (IV) and gardoside meathyl ester (V) as well as the lignan glucoside dehydrodiconiferyl alcohol-4-*O*-β-D-glucoside (VI) were isolated from the overground parts of *Bellardia trixago.* The structure elucidation of the isolated compounds (I-VI) was realized on the basis of spectral evidence (UV, IR, ^{1}H and ^{13}C NMR) and the results were confirmed by comparison of the isolated compounds with authentic samples on TLC.

Key Words: *Bellardia trixago,* Scrophulariaceae, iridoid glucosides, lignan glucoside.

Introduction

Bellardia trixago (L.) All. (Scrophulariaceae) is native to all regions of Turkey and the only member of the genus *Bellardia,* known in the Turkish flora (1).

From other species occuring in Europe of the genus, iridoids, flavonoids and diterpenes have been isolated (2-5).

In this study, the secondary metabolites of the above ground parts of *Bellardia trixago* were investigated.

Materials and Methods

Material: The above ground parts of *Bellardia trixago* were collected around of Alaçatı, in the province of Çeşme (İzmir) in April 1995. Voucher specimens have been deposited in the Herbarium of Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF-95013).

Extraction and Isolation: Dried and powdered above ground parts of the title plant (170 g) were extracted with methanol (2x1.5 l). The combined extracts (51.68 g) were evaporated under vacuum until dry. The residue was dissolved in water (500 ml) and the water soluble part was then succesively extracted with petroleum ether, chloroform and n-butanol. The n-butanol extract was evaporated to dryness with a yield of 13.6 g. After application of silica gel column chromatography with $CHCl_3$-MeOH-H_2O (80:20:1, 80:20:2 → 50:50:5) solvent systems, five main fractions (frs. A-E) were collected. After thin layer chromatographic (TLC) control of the fractions A-E in $CHCl_3$-MeOH-H_2O (80:20:2 and 61:32:7) solvent systems, frs. B-D were found to be worth of further study.

By subjecting Fraction D, eluted with $CHCl_3$-MeOH-H_2O (60:40:4), to medium-pressure liquid chromatography (MPLC) and elution with 15-25% MeOH, compound I (109 mg) was isolated in pure form. Compound II (58.8 mg), III (67.7 mg) and IV (87.7 mg) were obtained in similar manner, by subjecting fraction B, eluted with $CHCl_3$-MeOH-H_2O (80:20:2) to MPLC and elution with 25-40% MeOH. Finally, compound V (19.1 mg) and VI (6.4 mg) as well as IV (53.0 mg) were received from fraction C, eluted with $CHCl_3$-MeOH-H_2O (70:30:3), by application of MPLC and elution of 25-40% MeOH. Compounds isolated from the title plant are given in Figure 1.

Results

Aucubin (I)- UV λ max. (MeOH) 210 nm, IR (KBr) υ max. 3369, 2918, 1655, 1229, 1045 cm^{-1}. ^{1}H NMR (CD_3OD+DMSO-d_6, 300 MHz) δ 6.34 (dd, J=6.1/1.9 Hz,

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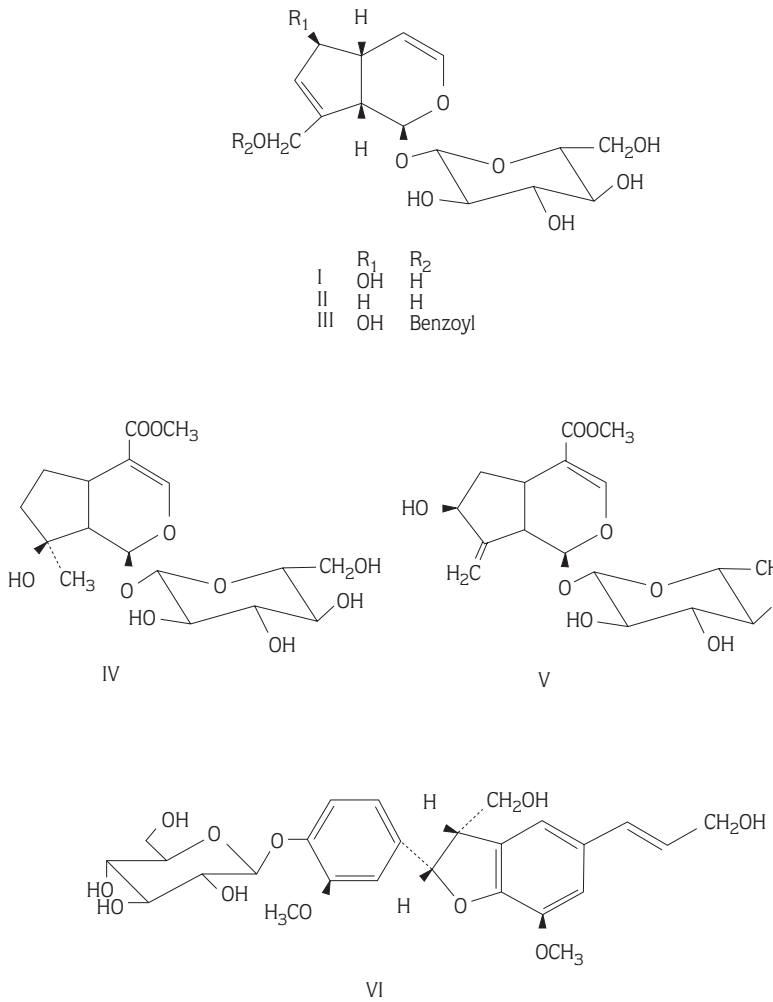

Figure 1. Iridoid and Lignan Glucosides Isolated from *Bellardia trixago*

H-3), 5.79 (br.s, H-7), 5.12 (dd, J=6.1/3.9 Hz, H-4), 4.98 (d, J=7.1 Hz, H-1), 4.70 (d, J=7.8 Hz, H-1'), 4.43 (m, H-6), 4.37 (d, J=15.4 Hz, H-10_A), 4.19 (d, J=15.4 Hz, H-10_B), 3.87 (dd, J=11.7/1.6 Hz, H-$6'_A$), 3.67 (dd, J=11.7/5.3 Hz, H-$6'_B$), 2.92 (dd (t), J=7.3 Hz, H-9), 2.68 (m, H-5).

Bartsioside (II)- UV λ max. (MeOH) 210 nm, IR (KBr) υ max. 3371, 2921, 1651, 1369, 1227 cm^{-1}. ^1H NMR (CD_3OD+DMSO-d_6, 300 MHz) δ 6.32 (dd, J=6.1/1.7 Hz, H-3), 5.77 (br.s, H-7), 5.18 (d, J=6.3 Hz, H-1), 4.95 (dd, J=6.1/3.6 Hz, H-4), 4.72 (d, J=7.8 Hz, H-1'), 4.33 (d, J=14.2 Hz, H-10_A), 4.20 (d, J=14.2 Hz, H-10_B), 3.89 (dd, J=11.9/1.5 Hz, H-$6'_A$), 3.70 (dd, J=11.9/5.3 Hz, H-$6'_B$), 2.97 (m, H-5), 2.82 (dd (t), J=7.8 Hz, H-9), 2.66 (m, H-6_A), 2.11 (m, H-6_B).

Melampyroside (III)- UV λ max. (MeOH) 273, 229, 209 nm, IR (KBr) υ max. 3401, 2924, 2360, 1717, 1602, 1375, 1279 cm^{-1}. ^1H NMR (CD_3OD+DMSO-d_6, 300 MHz) δ 8.09 (H-2"/6"), 7.65 (H-4"), 7.54 (H-3"/5"), 6.39 (dd, J=6.1/1.9 Hz, H-3), 5.90 (br.s, H-7), 5.15 (dd, J=6.1/3.9 Hz, H-4), 5.13 (d, J=15.3 Hz, H-10_A), 5.04 (d, J=7.3 Hz, H-1), , 4.99 (d, J=15.3 Hz, H-10_B), 4.73 (d, J=7.8 Hz, H-1'), 4.50 (m, H-6), 3.88 (dd, J=11.0/1.6 Hz, H-$6'_A$), 3.66 (dd, J=11.0/5.2 Hz, H-$6'_B$), 2.97 (m, H-5), 3.03 (br.t, H-9), 2.78 (m, H-5).

Mussaenoside (IV)- UV λ max. (MeOH) 237 nm, IR (KBr) υ max. 3393, 2925, 2360, 1697, 1638, 1439, 1303 cm^{-1}. ^1H NMR (CD_3OD+DMSO-d_6, 300 MHz) δ 7.36 (s, H-3), 5.41 (d, J=4.3 Hz, H-1), 4.62 (d, J=7.9 Hz, H-1'), 3.85 (dd, J=11.8/1.9 Hz, H-$6'_A$), 3.59 (dd, J=11.8/6.1 Hz, H-$6'_B$), 3.15 (m, H-5), 2.18 (dd, J=9.2/4.3, H-9), 2.23 (m, H-6_A), 1.67 (br.t, H-6_B), 1.67 (br.t, H-7_A), 1.39 (m, H-7_B), 1.28 (3H, s, CH_3-10).

Gardoside methyl ester (V)- UV λ max. (MeOH) 236 nm, IR (KBr) υ max. 3392, 2924, 2360, 1696, 1636, 1441, 1300 cm^{-1}. ^1H NMR (CD_3OD+DMSO-d_6, 300 MHz)

δ 7.47 (s, H-3), 5.46 (d, J=4.7 Hz, H-1), 5.38 (br.s, H-10), 4.68 (d, J=7.9 Hz, H-1'), 4.39 (br.t., H-7), 3.92 (dd, J=11.7/1.8 Hz, H-6'$_A$), 3.73 (dd, J=11.7/5.9 Hz, H-6'$_B$), 3.19-3.15 (m, H-9), 3.03 (m, H-5), 1.97 (2H, m, H$_2$-6).

Dehydrodiconiferyl alcohol-4-*O*-β-D-glucoside (VI)- UV λ max. (MeOH) 277, 216 nm, IR (KBr) υ max. 3392, 2925, 2360, 1601, 1516, 1457, 1267 cm^{-1}. ^{1}H NMR (CD$_3$OD+DMSO-d$_6$, 300 MHz) δ 7.20 (d, J=8.4 Hz, H-5), 7.09 (d, J=1.5 Hz, H-2'), 7.01 (2H, d, J=1.5 Hz, H-2/6), 7.00 (dd, J=8.5/1.7 Hz, H-6), 6.59 (d, J=15.9 Hz, H-α'), 6.30 (dt, J=15.9/5.8 Hz, H-β'), 5.64 (d, J=5.9 Hz, H-α), 4.95 (d, J=7.4 Hz, H-1'), 4.24 (dd, J=5.8/1.1 Hz, H-γ'), 3.94 (s, -OCH$_3$), 3.89 (s, -OCH$_3$/H-β), 3.80 (m, H-6'$_A$), 3.73 (m, H-6'$_B$), 3.68 (br.s, H-γ$_A$), 3.43 (br.s, H-γ$_B$). ^{13}C NMR (CD$_3$OD+DMSO-d$_6$, 75.5 MHz) δ 150.9 (C-3), 149.2 (C-4'), 147.7 (C-4), 145.5 (C-3'), 137.9 (C-1), 132.6 (C-5'), 131.8 (C-α'), 130.2 (C-1'), 127.9 (C-β), 119.4 (C-6), 117.8 (C-5), 116.5 (C-6'), 112.1 (C-2'), 111.3 (C-2), 102.5 (C-1"), 88.7 (C-α), 78.2 (C-3"), 77.9 (C-5"), 74.9 (C-2"), 71.3 (C-4"), 64.9 (C-γ), 63.8 (C-γ'), 62.4 (C-6"), 56.7 (2 x OCH$_3$), 55.3 (C-β).

Discussion

The structure elucidation of the isolated compounds (I-VI) was realized on the basis of spectral evidence (UV, IR, ^{1}H and ^{13}C NMR). The results were then confirmed by comparison of the compounds with authentic samples on TLC, by using CHCl$_3$-MeOH-H$_2$O (80:20:2 and 61:32:7) as solvent systems.

The UV spectra of the compounds I-III showed the characteristic absorptions of the enol-ether system at 210, 210 and 209 nm, confirmed by the bands observed at 1655, 1651 and 1602 (C=O) cm^{-1}, in the IR spectra (6). Additionally, the UV and IR absorptions of compound III at 273 nm and 1717 cm^{-1} revealed an esterification. In the ^{1}H NMR spectrum of compound I, the downfield shift for H-7 (δ 5.79, br.s) in addition to the absorptions for H-3 (δ 6.34, dd, J=6.1/1.9 Hz) and H-4 (δ 5.12, dd, J=6.1/3.9 Hz) revealed the presence of a second double bond in the structure of compound I. A doublet/triplet signal (J=7.3 Hz) at δ 2.92 attributed to H-9 clearly showed the position of this second double bond between C-7 and C-8. Two doublets appearing at δ 4.37 (J=15.4 Hz) and 4.19 (J=15.4 Hz) (AB system) indicated the presence of a secondary alcohol unit at C-8. Further on, the signal at δ 4.70 (d, J=7.8 Hz) is attributed to the anomeric proton of a β-D-glucose moiety.

Comparing the above findings to the other spectral data and those given in the literature (3,7,8), compound I was found to be aucubin.

The ^{1}H NMR spectrum of conpound II showed a significant relationship with that of aucubin (I) except for the signals for H-6. The two multiplet signals appearing at δ 2.66 and 2.11 clearly indicated the absence of any substitution at C-6. Therefore, by comparing the ^{1}H NMR spectral data of compound II with published data (3), it was found to be bartioside.

Similarly the ^{1}H NMR spectrum of compound III was also found to be identical with that of aucubin (I), with some additional signals which were clearly attributed to a benzoyl moiety. On the basis of the 0.8 ppm downfield shifts of the signals at δ 5.13 (d, J=15.3 Hz) and 4.99 (d, J=15.3 Hz) (attributed to H$_2$-10, AB system), compared to those of aucubin (I), it was suggested that the cyclopentanopyran moiety was esterified with a bezoic acid moiety on the secondary alcohol unit positioned at C-8. Therefore, the compound III was found to be melampyroside. These suggestions were also confirmed by published data (8-10).

The UV (237 and 236 nm, resp.), IR [$\upsilon_{max.}$ cm^{-1}: 1697 (C=O), 1638 (C=C) and 1696 (C=O), 1636 (C=C), resp.] and ^{1}H NMR data [δ 7.36 (s, H-3) and 3.65 (s, COOMe) and 7.47 (s, H-3) and 3.73 (s, COOMe), resp.] of compounds IV and V revealed the presence of the enol-ether group conjugated with COOMe.

Furthermore, the ^{1}H NMR spectrum of compound IV exhibited a singlet at δ 1.28 assignable to a tertiary methyl group attached to a hydroxyl bearing carbon, a doublet doublet (J=9.2 and 4.3 Hz) at δ 2.18 due to H-9, a doublet at δ 5.41 (d, J=4.3 Hz) arising from H-1, a multiplet at δ 3.15 due to H-5 and the signals arising from two methylene groups [δ 2.23 m and 1.67 br.t, H$_2$-6; 1.67, br.t and 1.39 m, H$_2$-7], as well as an anomeric proton signal at δ 4.62 (d, J=7.9 Hz). By comparing these findings with those of published data (8,10,11), compound IV was found to be mussaenoside.

In the ^{1}H NMR spectrum of compound V an olefinic proton signal at δ 5.38 (2H, br.s) showed the occurrence of an exo-methylene group in compound V. The presence of the signals attributed to H$_2$-6 protons at δ 1.97 (ABMX system) suggested that this exo-methylene is positioned at C-8. In addition, the ^{1}H NMR spectrum of compound V exhibited signals at δ 4.39 (br.t) assignable to a proton (H-7) attached to a hydroxyl bearing carbon, 5.46 (d, J=4.7 Hz) attributed to H-1, δ 3.03 (m) arising from H-5 and an anomeric proton signal at δ 4.68 (d, J=7.9 Hz). Therefore, the structure of compound V was found to be gardoside methyl ester. The suggested structure was

confirmed by the spectral data given in the literature (8,11,12).

The ^{1}H NMR spectrum of compound VI showed five aromatic protons, two olefinic protons (H-α' and H-β'), which appeared as an AB part of an ABX_2 system, two OMe groups and an anomeric β-D-glucose proton. Further confirmation of the structure of compound VI as well as the site of glucosidation was obtained from an analysis of its ^{13}C NMR spectrum, which showed signals corresponding to 18 carbon atoms (excluding the signals due to 2 x $O\underline{C}H_3$), consistent with a lignan structure, apart from the six signals due to β-D-glucose. No upfield shift of the signal of C-4 in compound VI, was observed as expected due to the glucosidation. However, significant downfield shifts of the signals of the *ortho*-(C-3 and C-5) and *para*- (C-1) related carbons were observed. Hereby the glucose moiety was located at C-4. Consequently, compound VI was found to be dehydrodiconiferyl alcohol-4-*O*-β-D-glucoside. This was also confirmed by published data (7).

In this study, the iridoid glucosides aucubin (I) and bartsioside (II), previously known from related taxa from Europea, were also isolated from *Bellardia trixago* growing in Turkey. However, other iridoid glucosides melampyroside (III), mussaenoside (IV) and gardoside methyl ester (V) as well as the lignan glucoside dehydrodiconiferyl acohol-4-*O*-β-D-glucoside (VI) were isolated for the first time from the genus *Bellardia.*

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