Iridoid Glycosides of *Leonurus persicus*

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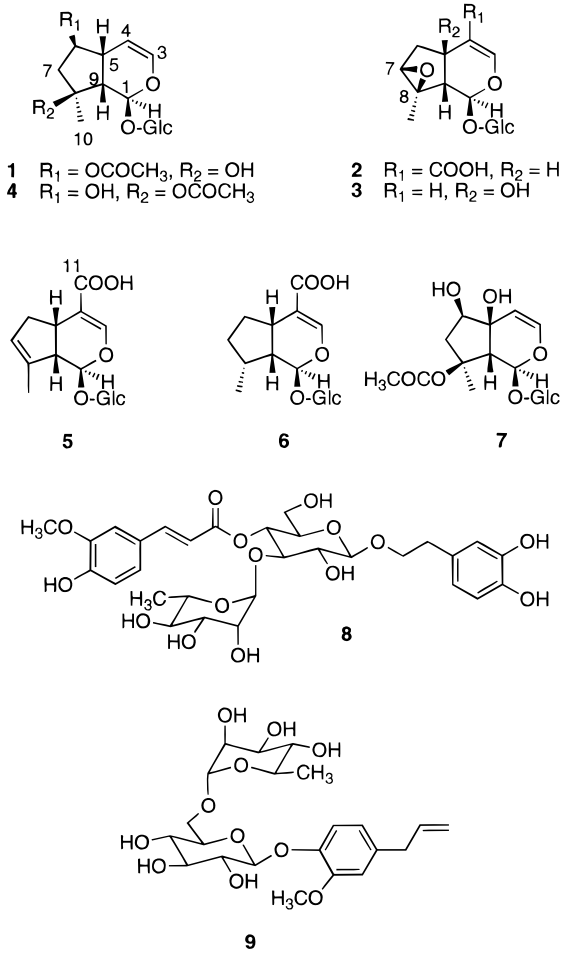
Two new iridoid glucosides, 6-*O*-acetylajugol (**1**) and 7,8-epoxy-8-*epi*-loganic acid (**2**), together with five known iridoid glucosides, galiridoside (**3**), ajugoside (**4**), 10-deoxygeniposidic acid (**5**), 7-deoxy-8-*epi*-loganic acid (**6**), and 8-*O*-acetylharpagide (**7**), have been isolated from the aerial parts of *Leonurus persicus*. Leucosceptoside A (**8**), eugenyl *β*-rutinoside (**9**), and kaempferol 3-*O*-glucoside (**10**) were also isolated. The structures of **1** and **2** were elucidated by extensive 1D- and 2D-NMR spectroscopy and molecular modeling. The structure of **3** was confirmed by single-crystal X-ray diffraction. Antimicrobial activity of compounds (**1**−**10**) was also evaluated against a panel of Gram-positive and Gram-negative bacteria and two strains of fungi.

Iridoids are of biogenetic and chemotaxonomic importance and are found mainly as glycosides in higher plants. Various biological activities are displayed by this class of compounds.[1−5] A bicyclic H-5/H-9 *β*,*β*-*cis*-fused cyclopentanopyran ring system was once considered to be a common structural feature of these substances. In 1984, unusual *Nepeta* iridoid glycosides[6] showing a different stereochemistry at C-1, C-5, and C-9 were isolated. In 1992, Foderaro et al.[7] isolated from *Penstemon secundiflorus* the first *trans*-fused iridoid glycoside, (5αH)-6-*epi*-dihydrocornin, whose structure was confirmed by a single-crystal X-ray analysis of the *p*-bromobenzoyl derivative. In *Nepeta* and *Penstemon* species, several enantiomeric iridoids have been found,[6−11] suggesting that iridoid glycosides have a more complex stereochemistry than first assumed.

As part of our ongoing investigations on the secondary metabolites of Turkish *Leonurus* species[12] (Lamiaceae), we have studied the iridoid glycosides of *Leonurus persicus*. Thirty-four compounds, of which twenty-six were new, comprising labdane and *seco*-labdane diterpenoids, two sterols and a flavone derivative, were recently isolated from the nonpolar extracts of *L. persicus*.[13−16] An additional study on the EtOAc extract of *L. persicus* has now resulted in the isolation of two new iridoid glucosides, 6-*O*-acetylajugol (**1**) and 7,8-epoxy-8-*epi*-loganic acid (**2**), together with five known iridoids, galiridoside (**3**), ajugoside (**4**), 10-deoxygeniposidic acid (**5**), 7-deoxy-8-*epi*-loganic acid (**6**), and 8-*O*-acetylharpagide (**7**). In addition, two phenylpropanoid glycosides, leucosceptoside A (**8**) and eugenyl *β*-rutinoside (**9**), as well as kaempferol 3-*O*-glucoside (**10**), were isolated. The structures of the novel iridoid glycosides (**1** and **2**) were determined by 1D and 2D NMR spectroscopy and molecular modeling while low-temperature X-ray crystallography was used to confirm the structure and stereochemistry of **3**.



Results and Discussion

A 4 g amount of the EtOAc extract (18.0 g), which was obtained from the air-dried aerial parts of *L. persicus* (900 g), were fractionated by vacuum-liquid chromatography (VLC) over a Si gel column, followed by ODS medium-pressure liquid chromatography (MPLC) to yield compounds **1**−**10**.

6-*O*-Acetylajugol (**1**) was isolated as an amorphous hygroscopic powder. The molecular formula was established as $C_{17}H_{26}O_{10}$ by LRFABMS (*m*/*z* 391 $[M + H]^+$) and ^{13}C NMR data. The FTIR spectrum showed absorption bands at 3400 (br OH), 1725 (ester), and 1665 cm^{-1} (C=C−O) and the UV spectrum exhibited maxima at 210 and

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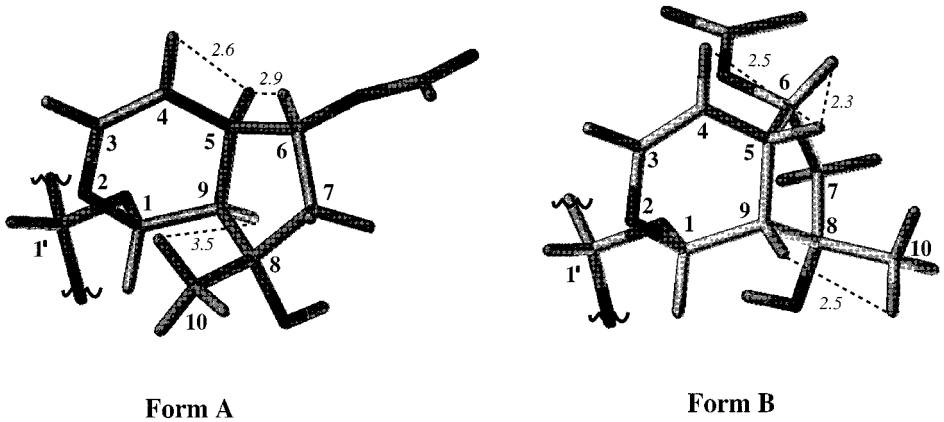

Figure 1. Graphical representation of the possible conformers of **1** (**A**, H-5/H-9 *cis*-*β*,*β*; **B**, H-5/H-9 *cis*-α,α) as derived from molecular modeling calculations showing important distances (Å) between NOE-interacting protons (see text). Note that the glycosidic part has been truncated at C-1′ for clarity as indicated by the wavy lines. Other distances of form **A** (**B**) not displayed in the figure are H-9/H-1 = 2.7 Å (2.4 Å); H-6/H-7 = 2.3 Å (2.3 Å); H-6/H-7′ = 3.0 Å (3.0 Å).

232 nm, indicating the presence of a nonconjugated enol−ether system and a saturated ester function. Analysis of the ^{1}H NMR spectrum revealed **1** to be an iridoid glucoside with an acetoxy (δ 2.04, s) and a tertiary methyl (δ 1.36, s) function. Signals in the region of δ 3.25−4.70 with a characteristic anomeric proton resonance at δ 4.66 (d, J = 7.8 Hz), as well as HMBC and NOE correlations between C-1/H-1′ and H-1/H-1′, suggested that **1** contains a β-glucopyranoside unit at the C-1 position of the aglycon. A complete interpretation of the remaining NMR data relied on the results of the DQF−COSY, HMQC, and HMBC experiments. Thus, the vicinally coupled olefinic protons at δ 6.19 (dd, J = 2.5, 6.3 Hz) and δ 4.92 (dd, J = 2.5, 6.3 Hz) were ascribed to H-3 and H-4, respectively, whose splitting pattern suggested C-5 to be unsubstituted. In the ^{1}H−^{1}H COSY spectrum, H-5 (δ 2.85, dd, J = 2.5, 9.1 Hz) correlated to H-9 (δ 2.51, dd, J = 2.5, 9.1 Hz), which in turn was scalarly coupled to H-1 (δ 5.47, d, J = 2.5 Hz). The absence of any other homonuclear coupling observed for H-9 was indicative of a totally substituted C-8. A prominent HMBC correlation from C-8 (δ_C 79.0, s) to the tertiary methyl signal (δ 1.36, s), in addition to heteronuclear long-range couplings between C-10/H$_2$-7 (see below), C-10/H-9, and C-8/H-9, showed the attachment of the methyl group at C-8. The chemical shift values of both C-8 and H$_3$-10 required a tertiary hydroxyl function to be at this position. The geminally coupled C-7 methylene protons (δ 2.18, dd, J = 6.5, 14.2 Hz, H-7α; δ 1.91, dd, J = 4.0, 14.2 Hz, H-7β) were mutually coupled to an oxymethine proton at 4.80 ppm (m), consistent with the acetoxy group being affixed to C-6 (δ_C 80.5, d). H-6 did not exhibit any ^{1}H−^{1}H COSY interaction with H-5, usually very characteristic for iridoids,[17] but the ^{1}H−^{13}C HMBC cross-peaks observed from C-6 to H-5 and H$_2$-7 secured its assignment. A final analysis of these data revealed that **1** has most of the structural features of ajugol,[18] which was also isolated from *Leonurus cardiaca*.[19] Compound **1** has an additional acetoxy function at the C-6 position causing downfield shifts of both H-5 and H-6 signals (δ [H-5] 2.85 in **1**, 2.72 in ajugol;[18] δ [H-6] 4.80 in **1**, 3.92 in ajugol[18]). From the comparison of the ^{13}C NMR data of **1** with those of 6-*O*-acetylmyoporoside,[20] which has an α-oriented acetoxy function at C-6, the 6-acetoxy group in compound **1** was tentatively assigned to have the β-orientation due to the abnormal downfield shift (+3 ppm) of C-5 and C-6. These data and additional NMR data of 6-*O*-acyl derivatives of ajugol[18] also suggested that the β-hydroxy group at C-6 was involved in the ester linkage.

To prove the relative stereochemistry of the chiral centers in **1**, a 2D ROESY experiment was performed. ROE cross-peaks of significant intensity between H-6α/H-7α, H-7α/H$_3$-10, and H$_3$-10/H-1 indicated that these H atoms lie on the same side (α) of the molecule. Therefore, the tertiary hydroxy and acetoxy functions must be in the β position. However, the observation of some ambiguous ROESY correlations (see Experimental Section) prompted us to run additional gradient-enhanced nuclear Overhauser effect spectroscopy (GOESY) in which well-separated resonances have been irradiated. The results of these experiments can be summarized as follows: (i) irradiation of H-1 produced enhancements on H$_3$-10, H-1′, and H-9; (ii) irradiation of H-9 resulted in a strong enhancement of H-5, indicating a *cis* configuration of both, but also had a considerable effect on H-1; (iii) when H-5 was irradiated H-4, H-9, as well as H-6 were found to be enhanced; (iv) irradiation of H-6 produced significant effects on H-7α and H$_3$-10 but also unexpected NOE effects on H-5 and H-7β.

Considering these correlations, two stereochemical arrangements of the cyclopentanopyran ring system are possible: H-5/H-9 *cis*-β,β and H-5/H-9 *cis*-α,α, with the usual (β) glycosidation pattern at C-1 (H-1α). To refine the conformation of the ring junction, a molecular modeling study using MOPAC, a semiempirical quantum chemical method, was performed. The heat of formation (ΔH_f) for two possible conformational ring isomers, form **A** (both H-5 and H-9 are β,β *cis*-oriented) and form **B** (both H-5 and H-9 are α,α *cis*-positioned) (Figure 1) was calculated by means of the AM1 minimization procedure. The results of modeling calculations showed no clear conformational preference for **1** as the difference of the heat of formation between form **A** and form **B** ($\Delta\Delta H_f$) was only 0.18 kcal/mol (ΔH_f = − 453.16 kcal/mol in **A**, ΔH_f = − 453.34 kcal/mol in **B**). The analysis of the relevant distances between the interacting hydrogens (Figure 1) was useful for the interpretation of the NOE/ROE signals. In form **B**, the interatomic distance between H-5 and H-6 (2.3 Å) is smaller than between H-4 and H-5 (2.5 Å). In form **A**, H-5 is spatially closer to H-4 (2.6 Å) than to H-6 (2.9 Å). These findings correspond with the observation of a stronger dipolar coupling (NOE) between H-4/H-5 than between H-5/H-6 in the ROESY spectrum. These geometric facts require a β-configuration at C-5 in form **A**. Using molecular modeling studies, it was possible to show that H-1 and H-9 in both stereoisomeric forms are separated by NOE-observable distances (2.7 Å in **A** and 2.3 Å in **B**). Indeed, a considerable dipolar coupling (NOE) between these protons

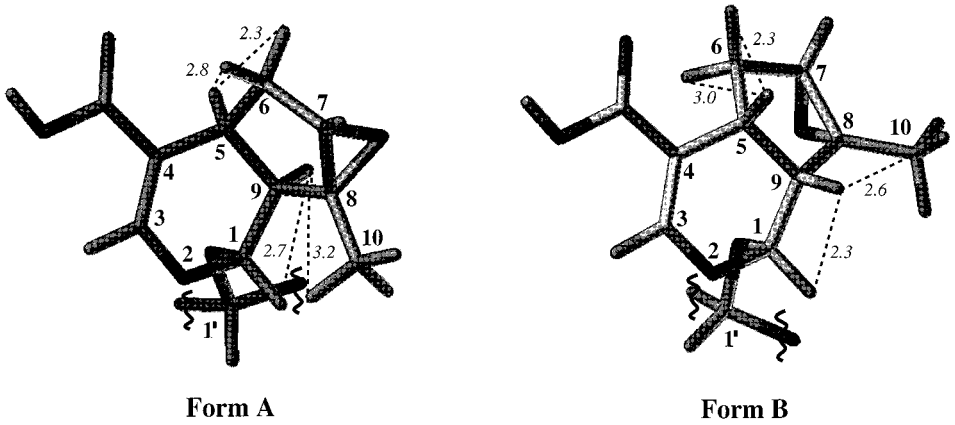

Figure 2. Graphical representation of the possible conformers of **2** (**A**, H-5/H-9 *cis*-β,β; **B**, H-5/H-9 *cis*-α,α) as derived from molecular modeling calculations displaying important distances (Å) between NOE-interacting protons. Again, the glycosidic part has been truncated at C-1′ for clarity.

was measured, indicating that these atoms are very close to each other. From the observations mentioned so far, it is impossible to distinguish between form **A** and form **B**. However, the absence of any NOE enhancement of H_3-10 upon irradiation of H-9 is only possible with a β-configuration at H-9 (form **A**), because only in this configuration the distance between these protons is large enough to justify the absence of the NOE. Similarly, the observed weak NOE correlation between H-6 and H-7β is in agreement with the calculated distance between these protons in forms **A** and **B** (Figure 1). Consequently, the measured NOE effects (Experimental Section) matched well all the proton−proton distances in the calculated structure **1A**, and, thus, we propose that **1** is 6-*O*-acetylajugol.

The molecular formula of compound **2** was determined as $C_{16}H_{22}O_{10}$ with six degrees of unsaturation, using a combination of LRFABMS (*m*/*z* 413 $[M + K]^+$) and ^{13}C NMR data. Its UV (λ_{max} 205 and 227 nm) and IR absorptions (ν_{max} 3450, 1685, and 1635, cm^{-1}) were consistent with the presence of an iridoid structure conjugated with a carboxyl function. The ^{13}C NMR spectrum of **2** showed 16 signals, six of which could be assigned to a β-glucopyranosyl moiety. The ^1H NMR signal at δ 7.20 (br s), assigned to H-3, suggested H-4 to be substituted, which was further strengthened by the typical ^{13}C NMR resonances associated with a C-4 carboxy-bearing iridoid in the region C-3 to C-5 (δ [C-3] 148.5, d; δ [C-4] 116.6, s; δ [C-5] 32.9, d), as well as the significant HMBC correlation from *C*OOH (δ 175.3) to C-3. The complete NMR data of **2** based on the $^1H-^1H$ and $^1H-^{13}C$ 2D NMR measurements were found to be similar to those of 7-deoxy-8-*epi*-loganic acid.[18,21] However, the H_3-10 methyl signal in the ^1H NMR spectrum of **2** was a singlet and showed a marked downfield shift (δ_H 1.60) compared with 7-deoxy-8-*epi*-loganic acid, supporting the presence of oxygenation at C-8. The chemical shift values of δ_C 65.8 (s), assigned as C-8, as well as δ_C 63.9 (d), assigned as C-7, were characteristic for a 7,8-epoxy function. Further evidence for the existence of an epoxy function at this position came from the homonuclear couplings between H-7/H_2-6 and H_2-6/H-5, in addition to ^2J and ^3J HMBC couplings from C-7 and C-8 to H_2-6, H-9, and H_3-10.

The configuration of the stereogenic centers in compound **2** was determined as described for compound **1**. The observation of the key ROE correlations for the noncontiguous protons H-1, H-6α, H-7, H_3-10, and H-1′ in the ROESY spectrum of **2** indicated that they were α-oriented and corroborated the β linkage of the glucose unit. Since no ROE was observed from H-7 to H-5, H-5 had to be on the opposite side (β) of the molecule. H-5 and H-9 showed a prominent ROE interaction, which suggested that they were *cis*-oriented. However, some unusual ROE signals were seen, namely, H-1/H-9, H-5/H-6α, and H-9/H_3-10, so that the possibility of an α,α-*cis*-fused ring system in **2** could not be excluded. Therefore, we decided to translate the results of the ROESY experiment into the molecular modeling program, as was done for compound **1**. Figure 2 illustrates 3D representations of the two proposed energy-minimized models, **A** and **B**. From the energetic point of view, both the **A** and **B** conformers have almost the same probability of existence ($\Delta H_f = -408.85$ kcal/mol for **A**, $\Delta H_f = -409.47$ kcal/mol for **B**, $\Delta\Delta H_f = 0.62$ kcal/mol), but a comparison of the interproton distances between H-1/H-9 and H-9/H_3-10 calculated by molecular modeling (Figure 2) and the intensity of the ROE correlations in the ROESY spectrum of **2**, clearly showed that the **A** conformer would be favored. Since an interatomic distance of 2.3 Å observed between H-5/H-6α in both conformers could also lead to an observable ROE effect, molecular modeling did not allow a complete discrimination between the two conformers in this case. We assigned the β position to H-5 on the basis of a comparison of the NMR data of **2** with those of related "common" iridoids,[18] as well as with the enantiomeric iridoid glycosides from *Nepeta* and *Penstemon* species.[6−11] The final confirmation of the determinations of the stereochemistry within **2** came from the single-crystal X-ray analysis of galiridoside (**3**), since repeated attempts to obtain suitable crystals from **2** were not successful. Galiridoside (**3**) is structurally very close to compound **2** and shows unusual ROE correlations very similar to those of **2** (see Experimental Section). A low-temperature X-ray diffraction experiment performed on **3** furnished a 3D ORTEP diagram (Figure 3) which fully supported the structure assigned earlier.[22] The structure of **2** corresponds with that shown for conformer **A** in Figure 2 and we propose the trivial name 7,8-epoxy-8-*epi*-loganic acid.

In addition to these new compounds, five known iridoid glycosides (**3**−**7**) have also been isolated in this study. Their planar structures were deduced by 1D and 2D NMR spectroscopy as well as by FABMS. The relative configurations of the chiral centers of **3**−**7** were established by examination of the Dreiding models, $[\alpha]^{20}_D$ values,[18] ^1H and ^{13}C NMR data, as well as by comparing their ROE data with those of compounds **1** and **2.** Additional information, including NOE/ROE and FABMS data of **3**−**7**, as well as the crystal structure of galiridoside (**3**, Figure 3), is given in the Experimental Section.

Three phenolic glycosides have also been isolated from *L. persicus*. Compounds **8** and **9** were identified as leucosceptoside A and eugenyl β-rutinoside, respectively, by

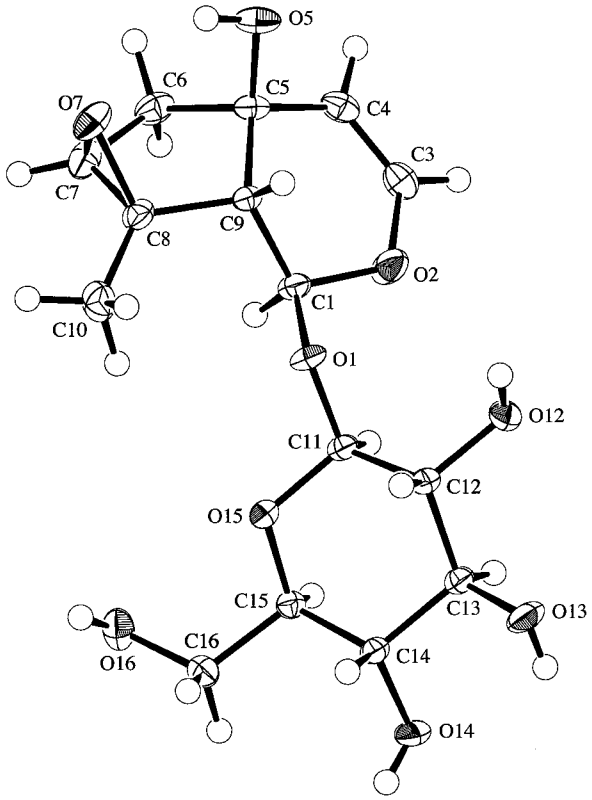

Figure 3. ORTEP[31] representation of galiridoside (**3**) (50% probability ellipsoids; H- atoms are given with arbitrary displacement parameters for clarity).

comparing their spectral data (1D and 2D NMR, FABMS) with those published in the literature.[23−25] To our knowledge, this is the second time that eugenyl β-rutinoside (**9**) has been isolated from a natural source.[24] This compound has also been artificially produced by cell cultures of *Eucalyptus perriniana* with eugenol as a substrate.[25] Compound **10** was characterized as kaempferol 3-*O*-glucoside by comparing its ^{1}H NMR data with previously published data[26] and by direct comparison of **10** with an authentic sample on a TLC plate.

Compounds **1**−**10** were tested for their antibiotic activity using a panel of Gram-positive and Gram-negative bacteria as well as two strains of fungi (see Experimental Section). Only leucosceptoside A (**8**) showed moderate antibacterial activity against *Staphylococcus epidermidis* at a concentration of 50 μg/spot.

The cyclopentanopyran ring system of the aglycon part of iridoid glycosides was once thought to be β,β-*cis*-fused universally, and, therefore, the stereochemistry was determined by the interpretation of the ^{1}H and ^{13}C NMR data, by chemical correlations and by analogy with compounds of known stereochemistry, assuming all iridoid glycosides to have this classical ring junction. Since the early 1980s, 1D or 2D NOE experiments have often been employed to determine stereochemistry. Nevertheless, the co-occurrence of both the usual (H-5/H-9 β,β-*cis*-fused) and enantiomeric iridoid aglycons in the same plant material (*Nepeta* and *Penstemon* sp.)[6−11] suggests that the iridoid glycosides are by no means metabolites of "known stereochemistry". The results of our molecular modeling experiments show that both *cis*-α,α- and *cis*-β,β-fused ring junctions of the aglycon with the usual sugar linkage (β) at C-1 have the same energetic probability of existence because there is only a small difference in the heats of formation; ranging between 0.2 and 0.6 kcal/mol. This work also illustrates that the stereochemical determination of solution conformations of iridoids by means of NOE/ROE data alone may lead to some controversy owing to the spatial proximity of pertinent protons of the ring system of the iridoid aglycon. Therefore, 1,2 NOE/ROE interactions should be evaluated very critically and replaced by 1,3 interactions whenever possible. Furthermore, stereochemical determinations should be supported by additional methods, such as molecular modeling, X-ray crystallography, and/or CD spectroscopy. Our results may also suggest that the early assignments for this class of compounds and even some recent iridoid publications in which NOE measurements and other techniques were not performed, should be viewed critically.

Concerning the iridoid glycosides of the genus *Leonurus*, only the isolation of galiridoside (**3**), ajugoside (=leonuride) (**4**), and ajugol[19] from *L. cardiaca* has been described previously. These substances contain an 8β-oxy-8α-methyl-type iridoid aglycon, which is characteristic for the Lamiaceae.[27] In addition to the new compound **1** and 8-*O*-acetylharpagide (**7**), the isolation of iridoids—substituted at C-4 by a COOH group (**2**, **5**, and **6**)—from *Leonurus* species is reported for the first time. These compounds may be of chemotaxonomic importance in the future.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 241 polarimeter using MeOH or H_2O as solvent. IR spectra were measured on a Perkin-Elmer 2000 FTIR spectrometer using pressed KBr disks. UV spectra were recorded in MeOH using an UVIKON 930 spectrophotometer. FABMS were measured on a ZAB 2-SEQ mass spectrometer in the positive mode using NOBA as the matrix. The NMR spectra were recorded at 27 °C on a Bruker AMX-300 spectrometer in CD_3OD or in D_2O at proton and carbon frequencies of 300 and 75.5 MHz, respectively. Multiplicities of the ^{13}C NMR spectra were determined from DEPT-135 data or ^{1}H,^{13}C HMQC spectra. In the ROESY experiments the spinlock was applied as a 300 ms cw-pulse of the amplitude $\gamma B_1/2\pi = 1500$ Hz (approximately 0.5SW). Silica used for vacuum-liquid chromatography (VLC) (column 6.5 × 20 cm, vacuum by H_2O aspiration) was TLC grade (Si gel $60F_{254}$, Merck, average particle size 15 μm). Medium-pressure liquid chromatographic (MPLC) separations were carried out with a Büchi 688 pump, a Büchi 684 fraction collector, and a Büchi MPLC column (46 × 2.6 cm, i.d.) including a precolumn. The column was packed with RP-18 material (Chromatographiegel C-18HL, 0.04−0.063 mm). All solvents were of HPLC grade.

Extraction and Isolation. A description of the plant material and of the extraction procedure has been published elsewhere.[13] An aliquot (4.0 g) of the EtOAc-soluble extract (18.0 g) was fractionated over Si gel (VLC) using a $CHCl_3$−MeOH−H_2O (9:1:0 to 6:40:0.1) gradient system to yield 21 fractions of 80 mL each. Three VLC fractions were subjected to further extraction employing H_2O−MeCN mixtures (0% to 15% MeCN). Galiridoside (**3,** 35 mg), ajugoside (**4,** 3 mg), 6-*O*-acetylajugol (**1**, 3 mg), and kaempferol 3-*O*-glucoside (**10**, 5 mg) were isolated from fraction 9 (329 mg). Purification of fraction 11 (299 mg) furnished 10-deoxygeniposidic acid (**5**, 8 mg), 7-deoxy-8-*epi*-loganic acid (**6**, 6 mg), 8-*O*-acetylharpagide (**7**, 17 mg), leucosceptoside A (**8**, 12 mg), and eugenyl β-rutinoside (**9**, 10 mg). Elution of the recombined VLC fractions 16−20 (239 mg) with pure H_2O yielded 7,8-epoxy-8-*epi*-loganic acid (**2**, 6 mg).

6-*O*-Acetylajugol (1): Amorphous, hygroscopic powder; $[\alpha]^{20}_D$ −103.5° (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.32) and 232 (4.31) nm; IR ν_{max} (KBr) 3400, 1725, 1665 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 5.47 (1H, d, J = 2.5 Hz, H-1), 6.19 (1H, dd, J = 2.5, 6.3 Hz, H-3), 4.92 (1H, dd, J = 2.5, 6.3 Hz, H-4), 2.85 (1H, dd, J = 2.5, 9.1 Hz, H-5), 4.80 (1H, m, H-6), 2.18 (1H, dd, J = 6.5, 14.2 Hz, H-7α), 1.91 (1H, dd, J = 4.0,

14.2 Hz, H-7β), 2.51 (1H, dd, J = 2.5, 9.1 Hz, H-9), 1.36 (3H, s, H_3-10), 2.04 (3H, s, OCOCH_3), 4.66 (1H, d, J = 7.8 Hz, H-1′), 3.19 (1H, dd, J = 7.8, 9.0 Hz, H-2′), 3.26−3.40 (3H, m, H-3′-5′), 3.65 (1H, dd, J = 5.6, 12.0 Hz, H-6′a), 3.89 (1H, dd, J = 1.9, 12.0 Hz, H-6′b); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 93.4 (d, C-1), 141.0 (d, C-3), 104.6 (d, C-4), 39.2 (d, C-5), 80.5 (d, C-6), 47.8 (t, C-7), 79.0 (s, C-8), 51.6 (d, C-9), 25.9 (q, CH_3-10), 21.1 (q, OCOCH_3), 172.8 (s, OCOCH$_3$), 99.4 (d, C-1′), 74.8 (d, C-2′), 78.1 (d, C-3′), 71.8 (d, C-4′), 78.3 (d, C-5′), 62.9 (t, C-6′). ROE data (w, weak; m, moderate; s, strong): H-1/H-9 w, H-1/H_3-10 m, H-1/H-1′ s, H-3/H-4 m, H-4/H-5 s, H-5/H-6 w, H-5/H-9 s, H-6/H-7α m, H-6/H-7β w, H-6/H_3-10 w, H-7α/H_3-10 m, H-7β/H-9 w, H-7β/H_3-10 w, H-1′/H-2′ m. LRFABMS m/z [M + H]$^+$ 391 (97), [M − HOAc + H]$^+$ 331 (24), [M − HOAc]$^+$ 330 (34).

7,8-Epoxy-8-*epi*-loganic acid (2): white amorphous powder; $[\alpha]^{20}_D$ −40° (c 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.07) and 227 (4.16) nm; IR ν_{max} (KBr) 3450, 1685, 1635 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 5.12 (1H, d, J = 9.5 Hz, H-1), 7.20 (1H, br s, H-3), 2.80 (1H, m, H-5), 1.41 (1H, dd, J = 10.4, 13.6 Hz, H-6α), 2.60 (1H, dd, J = 7.5, 13.6 Hz, H-6β), 3.28 (1H, m, H-7), 2.19 (1H, dd, J = 7.4, 9.5 Hz, H-9), 1.60 (3H, s, H_3-10), 4.81 (1H, d, J = 7.9 Hz, H-1′), 3.19−3.44 (4H, m, H-2′-5′), 3.64 (1H, dd, J = 5.8, 11.9 Hz, H-6′a), 3.90 (1H, dd, J = 1.9, 11.9 Hz, H-6′b); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 95.6 (d, C-1), 148.5 (d, C-3), 116.6 (s, C-4), 32.9 (d, C-5), 36.2 (t, C-6), 63.9 (d, C-7), 65.8 (s, C-8), 45.4 (d, C-9), 18.1 (q, CH_3-10), 175.3 (s, COOH), 99.9 (d, C-1′), 75.1 (d, C-2′), 78.0 (d, C-3′), 71.8 (d, C-4′), 78.6 (d, C-5′), 63.0 (t, C-6′). ROE data: H-1/H-9 m, H-1/H-6α s, H-1/H_3-10 s, H-1/H-1′ s, H-5/H-6α m, H-5/H-9 s, H-6α/H-1 s, H-6α/H-7 s, H-7/H_3-10 s, H-9/H_3-10 m, H-1′/H-2′ m. LRFABMS m/z [M + K]$^+$ 413 (85), [M + Na]$^+$ 397 (56).

Galiridoside (3): white amorphous powder; NMR data and $[\alpha]^{20}_D$ data were identical with those reported previously.[22,28] ROE data, H-1/H-6 w, H-1/H-9 m, H-1/H_3-10 s, H-1/H-1′ s, H-3/H-4 m, H-4/H-6 s, H_2-6/H-1 m, H_2-6/H-7 s, H-7/H_3-10 s, H-9/H_3-10 m, H-1′/H-2′ m. LRFABMS m/z [M + Na]$^+$ 369 (100).

Ajugoside (4): white amorphous powder; NMR data and $[\alpha]^{20}_D$ data were identical with those reported previously.[18,19] ROE data: H-1/H-9 m, H-1/H_3-10 s, H-1/H-1′ s, H-3/H-4 m, H-4/H-5 w, H-4/H-6 m, H-5/H-6 m, H-5/H-9 m, H-6/H-7α m, H-6/H-7β w, H-7α/H_3-10 m, H-7β/H_3-10 w, H-1′/H-2′ m. LRFABMS m/z [M + Na + H]$^+$ 413 (36).

10-Deoxygeniposidic acid (5): white amorphous powder; NMR and $[\alpha]^{20}_D$ data were identical with those published by Inoue et al.[29] NOE data: H-1/H-6α m, H-1/H-9 s, H-1/H_3-10 s, H-1/H-1′ s, H-5/H-9 s, H-5/H_3-10 m, H-6α/H-1 m, H-6α/H-7 s, H-6α/H-9 w, H-7/H-9 w, H-7/H_3-10 s, H-9/H_3-10 s, H-1′/H-2′ m. LRFABMS m/z [M + K]$^+$ 397 (42), [M + Na]$^+$ 381 (39).

7-Deoxy-8-*epi*-loganic acid (6): white amorphous powder; ^{1}H NMR spectra were recorded in CD_3OD and D_2O, separately, and compared to those described in the literature.[30] ^{13}C NMR (CD_3OD) and $[\alpha]^{20}_D$ data were identical with published data.[6,21] ROE data: H-1/H-9 m, H-1/H_3-10 m, H-1/H-1's, H-5/H-9 m, H-7α/H_3-10 s, H-8/H_3-10 s, H-1′/H-2′ m. LRFABMS m/z [M + K]$^+$ 399 (13), [M + Na]$^+$ 383 (57), [M + H]$^+$ 361 (12), [M − COOH]$^+$ 315 (13).

8-*O*-Acetylharpagide (7): white, amorphous powder; NMR and $[\alpha]^{20}_D$ data were identical with published data.[18] ROE data: H-1/H-9 m, H-1/H_3-10 s, H-1/H-1′ s, H-3/H-4 m, H-4/H-6 w, H-5/H-9 m, H-1′/H-2′ m. LRFABMS m/z [M + Na + H]$^+$ 429 (100), [M − HOAc + Na]$^+$ 369 (17).

Leucosceptoside A (8): yellowish, amorphous powder; NMR and FABMS data were identical with published data.[23]

Eugenyl β-rutinoside (9): pale, yellow amorphous powder; UV, NMR, FABMS, and $[\alpha]^{20}_D$ data were identical with data previously reported.[24,25]

Kaempferol 3-*O*-glucoside (10): yellowish, amorphous powder; ^{1}H NMR data were identical with those described in the literature;[26] TLC comparison with the original substance using three different solvent systems has further proved its identity.

Molecular Modeling. Structures were built and displayed using the molecular modeling program SYBYL (Version 6.3, Tripos Inc., St. Louis, MO, on a Silicon Graphics INDIGO2 EXTREME). Minimization was performed by means of MOPAC, a program for semiempirical calculations. AM1 was used as the Hamiltonian. The resulting conformations were analyzed by measuring H−H distances (Å).

Single-Crystal X-ray Analysis of 3.[32] A crystal with the molecular composition $C_{15}H_{22}O_9$, obtained by slow crystallization from MeCN−H_2O−MeOH, was used for a low-temperature X-ray structure determination. All measurements were made on a Rigaku AFC5R diffractometer using graphite-monochromated Mo Kα radiation (λ = 0.710 69 Å) and a 12 kW rotating anode generator. The intensities were collected using $\omega/2\theta$ scans, and three frequently measured standard reflections remained stable throughout the data collection. The intensities were corrected for Lorentz and polarization effects but not for absorption. The space group was determined from the systematic absences and packing considerations. The structure was solved by direct methods using SHELXS86,[33] which revealed the positions of all non-hydrogen atoms. The non-hydrogen atoms were refined anisotropically. All of the H atoms were located in a difference electron density map and their positions were allowed to refine together with individual isotropic displacement parameters. Refinement of the structure was carried out on F using full-matrix least-squares procedures, which minimized the function $\Sigma w(|F_o| - |F_c|)^2$, where $w = [\sigma^2(F_o) + (0.005F_o)^2]^{-1}$. A correction for secondary extinction was applied. A view of the molecule is shown in Figure 3. All calculations were performed using the teXsan crystallographic software package.[34] The absolute configuration has not been determined. The enantiomorph used in the refinement was based on the known configuration of the glucoside moiety.

Biological Assays. The antimicrobial potential of compounds **1**−**10** was tested against Gram-positive bacteria (*Bacillus cereus*, *B. aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Mycobacterium fortuitum*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), as well as against two strains of fungi (*Penicillium oxalicum*, *Candida albicans*), using the Agar-overlay method.

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Supporting Information Available: Table of crystallographic data for galiridoside (**3**), a table of fractional atomic coordinates and equivalent isotropic displacement parameters for **3**, and a figure showing the results of a 1D NOE experiment performed on compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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