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IRIDOID GLYCOSIDES FROM *GALIUM VERUM**

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Key Word Index—*Galium verum*; Rubiaceae; iridoid glycosides; *p*-hydroxyphenylpropionyloxy group; 3,4-dihydro-3-methoxy-asperuloside.

Abstract—From the aerial parts of *Galium verum* in flower, asperuloside and V_1 iridoid were isolated. From the mother liquor obtained on recrystallization of asperuloside, V_3 iridoid and daphylloside were also obtained.

INTRODUCTION

The following iridoid glycosides have been isolated from the herb *Galium verum* L.: asperuloside [1–4, 6], monotropein [4–6], scandoside, deacetylasperulosidic acid, geniposidic acid and asperulosidic acid [6]. We now report on the isolation and structure elucidation of **2** and **6** from the same source.

RESULTS AND DISCUSSION

Asperuloside (**1**) and daphylloside (**7**) were identified by their physical constants as well as by UV, IR and ^{1}H NMR spectroscopy [7, 8].

V_1 iridoid (**2**), colourless crystals, gave a blue colour reaction with Trim–Hill reagent [9]. Its UV and IR spectra are in accord with the presence of a conjugated iridoid enol–ether system, a γ-lactone and an aromatic ring respectively. The ^{1}H NMR spectrum (Table 1) is similar to that of asperuloside (**1**) apart from the following differences. There is no acetyl signal but an AABB system appears between δ6.7 and 7.2, indicating a *p*-disubstituted benzene derivative. In addition, a set of signals integrating for four hydrogens and characteristic of an A_2B_2 system appears between δ2.60 and 3.00. According to the chemical shift and signal form of the 2H-10 protons, the compound is acylated at this point.

The spectroscopic investigations were supported by some chemical reactions, too. Thus acetylation of compound **2** (acetic anhydride–pyridine) afforded the penta-acetate **3**. The ^{1}H NMR spectrum of **3** indicated the presence of four acetyl groups of alcoholic origin and one acetyl group of phenolic origin. Catalytic hydrogenation (Pd/C) of **2** gave compound **4** proving the allylic position of the *p*-hydroxyphenylpropionyloxy group. Methanolysis of **2** carried out in the presence of catalytic amount of sodium methoxide yielded compound **5** [10] which, on the basis of UV, IR and ^{1}H NMR data is identical with the product obtained by methanolysis of **1**.

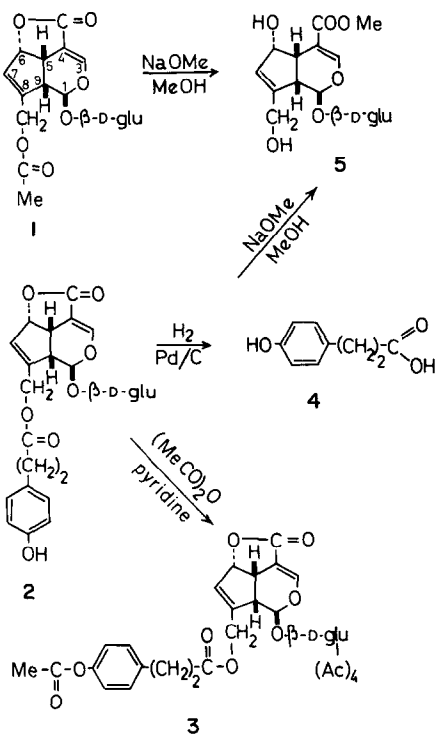


Fig. 1. Chemical transformations of **1** and **2**.

Thus, on the basis of chemical reactions and the data given in Table 1, compound **2** is an iridoid glycoside related to asperuloside (**1**) but carrying a *p*-hydroxyphenylpropionyloxy instead of an acetoxy group at C-10.

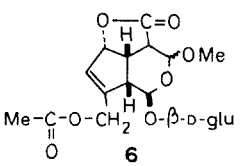
V_3 iridoid (**6**) and daphylloside (**7**) [8] were isolated from the mother lye of asperuloside after repeated purification by CC. V_3 iridoid (**6**) is a colourless amorphous substance, which does not absorb UV radiation above 210 nm, i.e. the characteristic enol–ether absorption is missing. In the ^{1}H NMR spectrum

*Dedicated to Professor Otto Clauder on his 75th birthday.

Table 1. ^{1}H NMR data of compounds **1**, **2**, **5** and **6** (100 MHz, 50°, D_2O, DSS as int. standard)

	Compound			
	1	**2**	**5**	**6**
H-1	6.04 *d* $J_{1,9}$ = 1.5 Hz	5.86 *d* $J_{1,9}$ = 1.5 Hz	5.07 *d* $J_{1,9}$ = 8.0 Hz	5.35 *d* $J_{1,9}$ = 4.5 Hz
H-3	7.43 *d* $J_{3,5}$ = 2.0 Hz	7.30 *d* $J_{3,5}$ = 2.0 Hz	7.72 *d* $J_{3,5}$ = 1.5 Hz	5.08 *d* $J_{3,4}$ = 4.5 Hz
H-6	5.81 *dd* $J_{6,5}$ = 7.0 Hz $J_{6,7}$ = 2.0 Hz	5.57 *dd* $J_{6,5}$ = 7.0 Hz $J_{6,7}$ = 2.0 Hz	4.91 *br d*	5.61 *dd* $J_{6,5}$ = 6.0 Hz $J_{6,7}$ = 2.0 Hz
H-7	5.92 *br s*	5.53 *d* $J_{7,6}$ = 2.0 Hz	6.10 *br s*	6.12 *d* $J_{7,6}$ = 2.0 Hz
2H-10	4.86 *br s*	4.66 *br s*	4.42 *br dd* J_{AB} = 14.5 Hz	4.90 *br s*
H-1′	4.96 *d* $J_{1',2'}$ = 7.5 Hz	4.79 *d* $J_{1',2'}$ = 7.0 Hz	4.89 *d* $J_{1',2'}$ = 7.5 Hz	4.83 *d* $J_{1',2'}$ = 7.5 Hz
Other groups	2.25 *s* –COMe	7.10 *d** 6.80 *d* ^3J = 8.0 Hz 2.60–3.00 A_2B_2 system	3.85 *s* –OMe	2.22 *s* –COMe 3.59 *s* –OMe

*p-Hydroxyphenylpropionyl.



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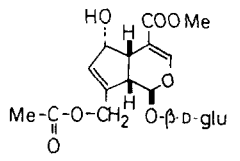

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(Table 1) only one signal for an olefinic proton (H-7) is present. The chemical shift of H-3 is in accord with the site of the methoxy group but its steric position cannot be interpreted unequivocally on the basis of its coupling constant. The lactone ring is intact and the compound is acylated at C-10. Compound **6** was also obtained by addition of methanol on asperuloside in the presence of a catalytic amount of methanolate in methanol.

According to these data, compound **6** is 3, 4-dihydro-3-methoxyasperuloside. Concerning the origin of **6** and **7**, it has been demonstrated by Inouye *et al.* [11] that **7** is a product of recrystallization of asperuloside in methanol. Though in our case **6** and **7** have been detected by TLC in the asperuloside fraction before recrystallization and according to our measurements [unpublished work] methanolysis of asperuloside is a rather slow process. However, it cannot be excluded that **6** and **7** are formed during chromatography of the plant extracts.

EXPERIMENTAL

CC was performed on Si gel (70–230 mesh, Merck), volatile materials were removed under red. pres. Mps are uncorr.

Isolation of iridoid fraction. Galium verum L. was collected in the garden of the Botanical Research Institute, Hungarian Academy of Sciences, Vácrátót and identified by Professor Imre Máthé (Research Institute of the Hungarian Academy of Science, Vácrátót, Hungary).

The fresh aerial part of the plant (1 kg) was chopped into small pieces and extracted with 70% aq. Me_2CO (4 l.). The Me_2CO extract was concd to an aq. suspension which was filtered, washed with $CHCl_3$ (200 ml), then chromatographed on 250 g Al_2O_3 (Woelm neutral) in H_2O. After evaporation of the H_2O the iridoid fraction (25 g) was chromatographed on 300 g Si gel in $CHCl_3$–MeOH (10 : 1) and divided into fraction I (1 g) and fraction II (10 g). Fraction II was chromatographed on 60 g polyamide (Serva) in H_2O to afford an asperuloside-containing fraction (8 g) and a mixture of **1** and **2** (1.5 g). Rechromatography of the mixture on 15 g polyamide gave 800 mg **2**.

V_1 iridoid (**2**) crystallized from MeOH, mp 118–120°. $[M]_{546}^{22} = -920°$ (MeOH; *c* 2.2%); UV λ_{max}^{EtOH} nm (log ε): 226 (4.14), 280 (3.17); IR ν_{max}^{KBr} cm^{-1}: 3360–3000, 1740, 1657, 1610, 1596, 1520; ^{1}H NMR: see Table 1.

Isolation of asperuloside (**1**), *daphylloside* (**7**) *and V_3 iridoid* (**6**). Repetition of the extraction procedure (×6) gave 50 g asperuloside fraction, which was crystallized from MeOH. The mother lye was evaporated (5 g) and chromato-

graphed on 150 g Si gel in Me_2CO–C_6H_6 (2:1) to give fractions: A, **6** and **1** (700 mg), B, **1** (3 g), C, **7** and **1** (1.3 g).

Fraction C was rechromatographed twice on Si gel in Me_2CO–C_6H_6 (3:2) to give 400 mg **7**; IR and ^{1}H NMR spectra superimposable on those of daphylloside. Fraction A was rechromatographed on Si gel in Me_2CO–C_6H_6 (3:2), then in $CHCl_3$–MeOH (10:1) to give 60 mg **6**.

V_3 iridoid (**6**). $[M]_{546}^{24} = -197.5°$ (MeOH, *c* 1.26%); IR ν_{max}^{KBr} cm^{-1}: 3600–3000, 1690, 1635; ^{1}H NMR: see Table 1.

10-Deacetyldaphylloside (**5**) *from* **1**. **1** (207 mg, 0.0005 M) in MeOH (3 ml) was treated with a soln of 1 M methanolic NaOMe (25 μl, 0.000025 M) at room temp. for 2 hr, then neutralized with HOAc. The residue after evaporation of the solvent was chromatographed on Si gel (20 g) in CH_2Cl_2–MeOH–H_2O (32:8:0.9), to give pure **5** (140 mg) which was crystallized from MeOH. Mp 136–139°; $[M]_{546}^{23} = +108.7°$ (MeOH, *c* 0.52%); UV λ_{max}^{EtOH} nm (log ϵ): 236 (3.92); IR ν_{max}^{KBr} cm^{-1}: 3600–3000, 1695, 1637; ^{1}H NMR: see Table 1.

10-Deacetyldaphylloside (**5**) *from* **2**. **2** (259 mg, 0.0005 M) in MeOH (3 ml) was treated with NaOMe and worked-up in the same way as **1**. The product was identical with **5** (mixed mp 136–139° and UV, IR and ^{1}H NMR).

p-Hydroxyphenylpropionic acid (**4**) (*phloretic acid*) *from* **2**. **2** (200 mg) in MeOH (6 ml) was added to 50 mg 10% Pd–C previously suspended in MeOH (3 ml) and satd with H_2. *Ca* 2.5 mol H_2 was absorbed over 80 min and then the catalyst was removed by filtration and washed several times with MeOH. The combined washings were evaporated to give an amorphous residue which was chromatographed on Si gel (20 g) in MeOH–Et_2O (1:20) to give 40 mg pure **4** which was crystallized from Et_2O, mp 129–130°, and proved to be identical with phloretic acid **4** (mmp 129–130°, and IR and ^{1}H NMR).

Acetylation of **2**. Compound **2** (100 mg) was treated with dry pyridine (0.5 ml) and Ac_2O (1.0 ml) for 16 hr at room temp. After addition of MeOH (3 ml) the soln was allowed to stand for 20 min, then evaporated to give an amorphous residue (150 mg). Chromatography of this residue on Si gel (20 g) in Et_2O, gave pure **3** (90 mg) $[M]_{546}^{24} = -731.8$; ^{1}H NMR (100 MHz, $CDCl_3$): δ 5.75 (2H, *br s*, H-1, H-7), 7.24 (1H, *d*, $J_{3,5} = 2.0$ Hz, H-3), 5.54 (1H, *br d*, $J_{6,5} = 7$ Hz, H-6), 4.72 (2H, *br s*, H-10), 2.77–3.17 (4H, A_2B_2 system), 2.40 (3H, *s*, phenolic-acetyl), 2.17 (6H, *s*, acetyl), 2.12 (3H, *s*, acetyl), 2.10 (3H, *s*, acetyl).

3, 4-Dihydro-3-methoxy-asperuloside (*V_3 iridoid*, **6**) *and* **7** *from* **1**. **1** (1.24 g, 0.003 M) in MeOH (30 ml) was treated with a soln of 1 M methanolic NaOMe (75 ml, 0.000075 M) at −30° for 30 min, then neutralized with HOAc. The residue obtained after evaporation was chromatographed on Si gel (100 g) in MeOH–$CHCl_3$ (1:5). The first fraction afforded pure **6** (25 mg) which proved to be identical with the isolated compound **6** (IR and ^{1}H NMR). The second fraction from the Si gel column afforded pure **7** (800 mg) which proved to be identical in all respects with daphylloside.

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