8. Euphroside, A New Iridoid Glucoside from *Euphrasia salisburgensis* HOPPE[1])[2])

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Summary

Five iridoid glucosides have been isolated from the whole plant of *Euphrasia salisburgensis*. The structure of the new compound, named euphroside, and the identity of the others have been established by chemical transformations and spectral data.

Introduction. - The genus *Euphrasia* consists of about eighty species of which thirteen are native to Switzerland [2]. Earlier investigations on this *genus* are limited to the identification and/or isolation of aucubin [3] [4], catalpol [5] and melampyroside [6]. As a part of systematic isolation and structure determination of iridoid glycosides from various plants belonging to the family *Scrophulariaceae* we have now studied the glycosidic constituents of *Euphrasia salisburgensis*.

Results and discussion. - Fractionation of the methanolic extract of the whole plant of *Euphrasia salisburgensis* gave a new iridoid glucoside, euphroside (**3**), along with the known glucosides boschnaloside (**1**), ixoroside (**2**), aucubin (**6**) and geniposidic acid (**7**). The structure **3** for the new compound based upon the following facts: Euphroside (**3**) was obtained as an amorphous powder, $[\alpha]_D^{20} = -167.27°$ ($c = 0.63$, MeOH) with the molecular formula $C_{16}H_{24}O_{10}$. Its UV. spectrum showed λ_{max} at 237 nm assigned to a conjugated enol-ether function. The IR. spectrum (KBr) of **3** showed absorption at 3360 cm^{-1} (br., OH), 1670 cm^{-1} and 1635 cm^{-1} indicating the presence of an α,β-unsaturated aldehyde function. The ^{1}H-NMR. (D_2O) of euphroside (**3**) showed two singlets at δ 9.62 and 1.62 ppm assignable to an aldehyde and a methyl group such as $H_3C(10)$, respectively. Acetylation of **3** at room temperature with acetic anhydride and pyridine gave the tetraacetate **4** which showed signals for four acetyl groups and two tertiary hydroxyl groups (exchangeable with D_2O). Further acetylation of **4** at room temperature with acetic anhydride and 4-dimethylaminopyridine gave the pentaacetate **5**, the ^{1}H-NMR. of which showed signals for five acetyl groups and one tertiary hydroxyl group (exchangeable with D_2O). The signal arising from the methyl group at δ 1.40 ppm was shifted downfield (0.18 ppm) compared to the corresponding

[1]) Part 1 in the series 'Glycosides of *Euphrasia species*'.

[2]) See [1].

[3]) Part of the Ph. D. thesis of *O. Salama* to be submitted to the ETH-Zürich.

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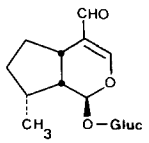
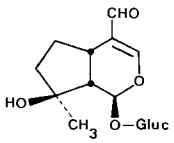
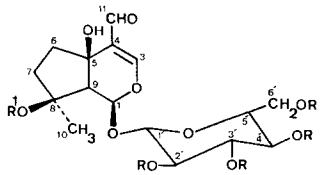


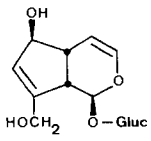
1 Boschnaloside



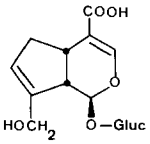
2 Ixoroside



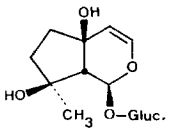
3 Euphroside; $R = R^1 = H$
4 Euphroside tetraacetate; $R = Ac$; $R^1 = H$
5 Euphroside pentaacetate; $R = R^1 = Ac$



6 Aucubin



7 Geniposidic acid



6-Deoxyharpagide

signal in **4**. From this observation it can be deduced that one of the hydroxyl groups is located at C(8) and the other one (not easily acetylable) at C(5) or C(9). The sharp singlet at δ 7.92 ppm assigned to the H–C(3) proton, highly deshielded by the presence of the carbonyl group at C(4), showed that C(5) must be substituted. The appearance of H–C(1) as doublet (coupling with H–C(9)) gave additional support for the above interpretation.

On going from the tetraacetate **4** to the pentaacetate **5** showed a deshielding of 0.5 and 0.3 ppm to H–C(1) and H–C(9) respectively and suggested HO_β–C(8) in euphroside [7].

Table. *^{13}C-NMR. data of boschnaloside, ixoroside, euphroside and 6-deoxyharpagide*[a])

C-Atom	Boschnaloside	Ixoroside	Euphroside	6-Deoxy-harpagide[c])
1	97.39 *d*	96.44 *d*	95.32 *d*	93.36 *d*
3	164.08 *d*	163.19 *d*	163.08 *d*	140.89 *d*
4	125.97 *s*	125.82 *s*	126.35 *s*	110.58 *d*
5	36.88 *d*	29.84 *d*	71.33 *s*	71.87 *s*
6	31.13 *t*	29.53 *t*	37.58 *t*	38.77 *t*
7	33.42 *t*	40.87 *t*	40.27 *t*	40.19 *t*
8	32.08 *d*	80.11 *s*	78.79 *s*	79.62 *s*
9	43.80 *d*	51.86 *d*	61.29 *d*	60.93 *d*
10	16.63 *qa*	24.54 *qa*	23.66 *qa*	24.71 *qa*
11	193.02 *d*	193.11 *d*	192.61 *d*	–
1′	99.72 *d*	99.76 *d*	99.75 *d*	99.16 *d*
2′	74.48 *d*	74.44 *d*	74.24 *d*	74.50 *d*
3′	78.12 *d*[b])	78.12 *d*[b])	78.27 *d*[b])	78.18 *d*[b])
4′	71.44 *d*	71.42 *d*	71.51 *d*	71.72 *d*
5′	77.71 *d*[b])	77.71 *d*[b])	77.28 *d*[b])	77.58 *d*[b])
6′	62.78 *t*	62.73 *t*	62.72 *t*	62.80 *t*

[a]) The spectra were recorded in CD_3OD. Chemical shifts in ppm relative to internal TMS.
[b]) These values are interchangeable in the vertical column.
[c]) Data taken from [9].

Further confirmation of the proposed structure **3** as well as the stereochemical assignment is obtained from its ^{13}C-NMR. spectrum *(Table)* which showed 16 signals. The assignment of the signals is based on (i) multiplicity of the signals in the single frequency off resonance decoupled (SFORD) spectrum, (ii) literature data on iridoid glucosides [8] and (iii) comparison of the spectra of **1**, **2** and **3**. It has recently been reported that the shielding caused by a quaternary HO–C(8) group to C(9) and the chemical shift value of H_3C–C(10) are reliable guide in revealing the configuration at C(8) [9]. Thus comparison of the chemical shift values of euphroside with that of related compounds established the structure and configuration as given in the formula (**3**).

As stated above, *Euphrasia salisburgensis* also contains boschnaloside (**1**) and ixoroside (**2**) beside euphroside (**3**). Thus, it would be reasonable to presume that these glucosides are biosynthesized in the sequence **1** → **2** → **3** in the plant.

The co-occurrence of boschnaloside (**1**) and its mono- and dioxygenated analogs, ixoroside (**2**) and euphroside (**3**), respectively, is an unique feature of this plant. Interestingly all the three compounds have an aldehyde function at C(4) which is still rare in nature. These glucosides may serve as taxonomic markers for the *genus Euphrasia*.

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Experimental Part

General. Melting points were determined on a *Mettler* FP5/FP52 apparatus. UV. spectra [λ_{max}(logε)] were determined on a *Perkin-Elmer* 550 spectrometer. IR. spectra (cm^{-1}) were determined on a *Perkin-Elmer* 257 instrument. ^{1}H- and ^{13}C-NMR. spectra (δ ppm, J Hz) were obtained at 100 MHz using a *Varian* HA-100 spectrometer and at 25.2 MHz in *Fourier* transform mode using a *Varian* XL-100-12 spectrometer, respectively, using TMS as an internal standard. MS. (*m/z*) were recorded with a *Hitachi-Perkin-Elmer* RMU 6M spectrometer. Silica gel 60 (70–230 mesh, *Merck*) and neutral alumina (*Woelm* N, Act. 1) were used for column chromatography. Silica gel 60 F_{254} *(Merck)* prepared plates were used for TLC. Spots were detected by UV. fluorescence and spraying with vanillin/H_2SO_4 followed by heating at 120° for 5–10 min. A *Waters Assoc.* HPLC. model ALC 201 was used throughout. A *Waters Assoc.* M-6000 pump was used as the solvent delivery system and a U 6K septumless injector. The system was equipped with a *Perkin-Elmer* spectrophotometer (LC 55) with a variable wave length detector. For the analytical and the semi-preparative HPLC. work μ*Bondapak*-C_{18} column (30 cm × 3.9 mm I.D.) and *Knauer*-C_{18} (30 cm × 16 mm I.D.) column respectively, were used. For the preparative work a *Waters* Prep. LC/500 system equipped with a reversed phase column was used. For the preparative low pressure liquid chromatography a *Perpex Vario*-step pump was used.

Abbreviations: LC. = liquid chromatography, LPLC. = low pressure liquid chromatography, HPLC. = high pressure liquid chromatography, RT. = room temperature.

Extraction and purification. – Fresh whole plant of *Euphrasia salisburgensis* (180 g) collected from the *Flims* area, Switzerland (6100 foot) in September 1979, were cut into small pieces and extracted with MeOH at 40° (3 × 1 l). After concentration of the combined extracts *in vacuo*, H_2O (0.5 l) was added and the H_2O-insoluble material was removed by filtration through *celite*. The filtrate was extracted with petroleum ether (3 × 0.5 l) and the soluble part was rejected. The aqueous concentrate was filtered through a prewashed (H_2O) column of neutral Al_2O_3 (200 g) eluting with H_2O. The aqueous eluate was concentrated and lyophilized to give crude glucoside fraction (4.2 g).

A portion of the mixture (4 g) was chromatographed over silica gel (200 g), eluting with CH_2Cl_2/MeOH/H_2O 80:20:2 (2 l), 70:30:3 (1 l), 60:40:4 (1 l) and five fractions A (0.220 g), B (0.29 g), C (0.430 g), D (0.18 g) and E (1.7 g) were collected.

Boschnaloside (**1**). Fr. A (200 mg) which was subjected to semi-preparative HPLC. [MeOH/H_2O 45:55; flow rate 8 ml/min] gave pure **1** (170 mg), $[\alpha]_D^{20} = -142.13°$ ($c = 0.69$; MeOH). – UV., IR., ^{1}H-NMR. [10] and ^{13}C-NMR. *(Table)*.

Ixoroside (**2**). Fr. B on prep. LC. [MeOH/H_2O 15:85; flow rate 100 ml/min] gave pure **2** (220 mg), $[\alpha]_D^{20} = -140.66°$ ($c = 0.91$; MeOH). – UV., IR., ^{1}H-NMR. [7] and ^{13}C-NMR. *(Table)*.

Fr. C (420 mg) on prep. LC. [MeOH/H_2O 10:90; flow rate 100 ml/min] gave **3** (130 mg) and **6** (110 mg).

Euphroside (**3**). $[\alpha]_D^{20} = -167.27°$ ($c = 0.63$; MeOH). – UV. (EtOH): 237 (4.01). – IR. (KBr): ~3360 (br., OH), 1670 (α,β-unsaturated CHO), 1630 (C=C). – ^{1}H-NMR. (D_2O): 9.62 (*s*, 1H, H–C(11)); 7.90 (*s*, 1H, H–C(3)); 6.38 (*d*, $J < 1$, 1H, H–C(1)); 2.93 (*d*, $J < 1$, 1H, H–C(9)); 1.90–2.74 (*m*, 4H, 2 H–C(6) and 2 H–C(7)); 1.62 (*s*, 3H, 3 H–C(10)).

Euphroside tetraacetate (**4**). Euphroside (**3**) (60 mg) was acetylated with acetic anhydride/pyridine at RT. in the usual way and the product was recrystallized from ether to give **4** (35 mg) as fine needles, m.p. 209–210°, $[\alpha]_D^{20} = -125.94°$ ($c = 0.66$; $CHCl_3$). – UV. (EtOH): 242 (4.02). – IR. (KBr): ~3500 (br. OH), 1755 (C=O, ester), 1690 (α,β-unsaturated CHO), 1630 (C=C). – ^{1}H-NMR. ($CDCl_3$): 9.36 (*s*, 1H, H–C(11)); 7.10 (*s*, 1H, H–C(3)); 5.59 (*d*, $J = 3$, 1H, H–C(1)); 4.80–5.36 (4H, H–C(1′), H–C(2′), H–C(3′) and H–C(4′)); 4.22 (*m*, 2H, 2 H–C(6′)); 3.62–3.86 (*m*, 1H, H–C(5′)); 3.0 (br. *s*, 2H, HO–C(5) and HO–C(8)); 2.50 (*d*, $J = 3$, 1H, H–C(9)); 1.60–2.40 (4 CH_3COO, 2 H–C(6) and 2 H–C(7)); 1.23 (*s*, 3H, 3 H–C(10)). – MS.: 544 (M^+, no peak), fragment ion peaks at 214 ($M^+ - 331$) (1), with H transfer, 196 ($M^+ - (331 + H_2O)$) (13), 179 ($M^+ - (331 + H_2O - OH)$) (26), 178 ($M^+ - (331 + 2\,H_2O)$) (4), 168 ($M^+ - (331 + H_2O + OH)$) (10), 160 ($M^+ - (331 + 2\,H_2O)$) (6), 150 ($M^+ - (331 + 2\,H_2O + CO)$) (4), 132 ($M^+ - (331 + 3\,H_2O)$) (4) and due to the glucose part at 331 (48), 289 (2), 271 (6), 211 (4), 187 (4), 169 (70), 127 (10), 109 (40).

Euphroside pentaacetate (**5**). To a solution of **4** (30 mg) in acetic anhydride (2 ml), 4-dimethylaminopyridine (10 mg) was added and the mixture was allowed to stand at RT. for 18 h. Followed by the usual work-up gave a residue which was recrystallized from ethanol to give **5** (18 mg) as fine needles, m.p. 157–158°, $[\alpha]_D^{20} = -120.0°$ ($c = 0.82$, $CHCl_3$). – UV. (EtOH): 243 (4.30). – IR. (KBr): 3540 (OH), 1790 (C=O, ester), 1680 (α,β-unsaturated CHO), 1625 (C=C). – ^{1}H-NMR. ($CDCl_3$): 9.41 (*s*, 1H, H–C(11)); 7.17 (*s*, 1H, H–C(3)); 6.08 (*d*, $J = 2$, 1H, H–C(1)); 4.74–5.35 (4H, H–C(1′), H–C(2′), H–C(3′) and H–C(4′)); 4.04–4.46 (*m*, 2H, 2 H–C(6′)); 3.66–3.88 (*m*, 1H, H–C(5′)); 3.04 (br. *s*, 1H, HO–C(5)); 2.80 (br. *s*, 1H, H–C(9)); 1.90–2.40 (5 CH_3COO, 2 H–C(6) and 2 H–C(7)); 1.40 (*s*, 3H, 3 H–C(10)).

Aucubin (**6**) was found to be identical with an authentic sample by comparison of their IR. and ^{1}H-NMR. spectra.

Geniposidic acid (**7**). Fr. D which was subjected to LPLC. [MeOH/H_2O 10:90] gave **7** (75 mg), $[\alpha]_D^{20} = +20.3°$ ($c = 0.94$; MeOH). The identity of **7** was established by direct comparison [TLC., ^{1}H- and ^{13}C-NMR.] with authentic geniposidic acid [9] [11].

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