60. Saponins with Molluscicidal Activity from *Hedera helix* L.

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(10.III.80)

Summary

Four triterpenoid saponins (**1**–**4**) have been isolated from the berries of *Hedera helix* L. *(Araliaceae)* by droplet counter-current and preparative liquid chromatography. The structures have been established by FD./MS. and ^{13}C-NMR. spectroscopy. The isolated compounds were found to possess strong molluscicidal activity against the bilharziasis-transmitting snail *Biomphalaria glabrata.* Saponin **4**, identified as hederagenin 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside, is a new natural product.

Introduction. – Various attempts are presently made in order to control bilharziasis by killing the transmitter of this endemic disease. But there is still a need for continued screening for more selective and efficient molluscicides as the presently available compounds or formulations tend to be generally biocidal, affecting many of the plants or animals (or both) in the snail habitats [1]. Naturally occurring molluscicides isolated from various plant sources are currently receiving considerable attention since the discovery of very active saponins in the berries of *Phytolacca dodecandra* L'HÉRIT *(Phytolaccaceae)* [2–5].

In a previous work, we reported the isolation and structure determination of two active spirostanol glycosides from the bark of *Cornus florida* L. *(Cornaceae)* [6].

In connection with our systematic screening studies on compounds with molluscicidal activity from medicinal plants, we noticed that the crude methanol extract of *Hedera helix* L. (common ivy) berries killed *Biomphalaria glabrata* snails at concentrations as low as 40 ppm. The crude leaf extract was less active and thus, not investigated in the present study. In a preliminary communication dealing with the application of droplet counter-current chromatography (DCCC.) to the isolation of polar natural products, we mentioned the separation of the major saponins of common ivy berries [7]. In the present paper, we report an improved isolation procedure and the structure determination of the active principle.

Results. – The fresh berries (150 g) of *Hedera helix* L. were extracted with methanol. After concentration, this extract was partitioned between butanol and water. The butanol layer (1.2 g) exhibiting the biological activity was submitted to DCCC. using $CHCl_3$/MeOH/H_2O 7:13:8 as solvent by the descending method. Compounds **1** (89 mg) and **4** (115 mg) were obtained directly in a pure form,

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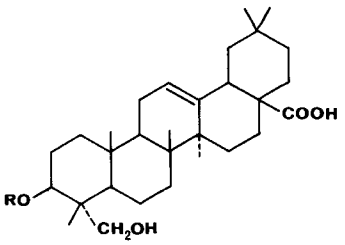

1 R = α-L-arabinopyranosyl
2 R = β-D-glucopyranosyl
3 R = α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl
4 R = β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl

whereas **2** and **3** were poorly separated. Separation of these two substances was achieved by preparative liquid chromatography on silica gel with $CHCl_3$/MeOH 17:3.

Acid hydrolysis of **1–4** afforded the same aglycone ($M^+ = 472$, $C_{30}H_{48}O_4$) identified as hederagenin [8] from MS. data of its methyl ester ($M^+ = 486$) [9] and by ^{1}H- and ^{13}C-NMR. spectroscopy [10]. The sugars obtained from the saponin hydrolysates were arabinose in **1**, glucose in **2** and **4** and arabinose and rhamnose in **3**. Field-desorption mass spectroscopy (FD./MS.) [11] carried out on the underivatized saponins, gave a molecular-ion for each compound (base peak $(M+H)^+$ or $(M+Na)^+$), namely 604, 634, 750, 796 for **1–4**, respectively. Thus, saponins **1** and **2** are monosaccharides whereas **3** and **4** possess each two sugar moieties. The FD./MS. of compound **4** is shown in the *Figure*. The fragments with *m/z* 797, 819 and 835 correspond to $(M+H)^+$, $(M+Na)^+$ and $(M+K)^+$. The weak peak at 635 is due to the loss of one glucose unit. Under the used experimental conditions, no aglycone peak could be observed. In the spectrum of saponin **3**, a fragment with *m/z* 605 corresponding to $[(M+H)-146]^+$, indicates that rhamnose is the terminal sugar. Furthermore, the sequence rhamnose-arabinose-hederagenin was confirmed by mild hydrolysis of **3** to the monoarabinoside **1**. In all the isolated saponins, the sugar is attached to the aglycone at position **3**. This was evidenced by ^{13}C-NMR. spectroscopy: C(3) of **1–4** appeared at 81.8–81.1 ppm whereas the chemical shift of this C-atom was 76.4 ppm in hederagenin [10].

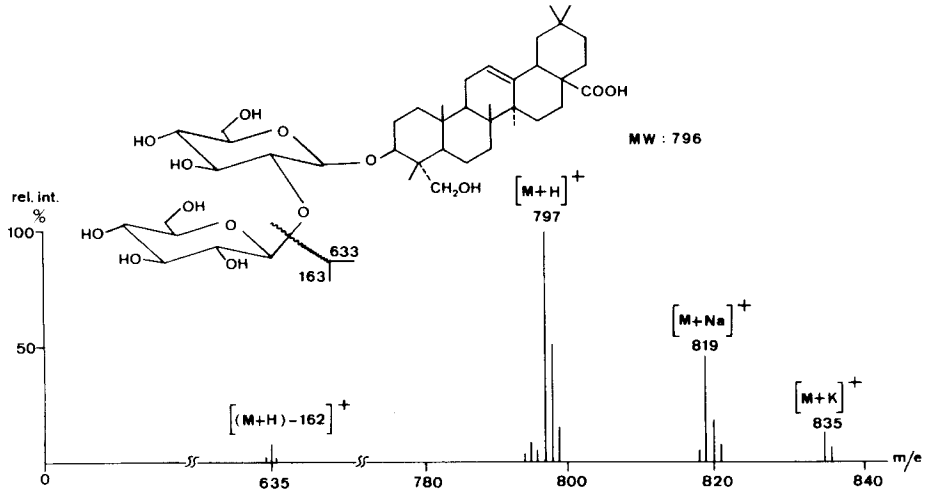

Figure. *FD./MS. of compound* **4** (Emitter current 20 mA)

Table. *^{13}C-NMR. data of the glucose moieties of saponin* **4**. Pertinent shifts discussed in the text are shown in italics.

C′ and C″	1	2	3	4	5	6
Inner moiety	*103.1*	*83.0*	*77.9*	70.8	77.6	62.1
Terminal moiety	*105.0*	*75.9*	*77.4*	70.8	77.4	62.1

^{13}C-NMR. spectroscopy was also used for the determination of the interglycosidic linkages. Assignment and chemical shifts of the sugar C-atoms of **4** are given in the *Table*. The anomeric C-signal at this relatively high field can be explained in terms of a substitution effect by another glycosyl linkage at C(2′) [12]. The glycosidic linkage at this position is further evidenced by the fact that the C(2′)-signal (inner glucose moiety) is shifted downfield by 7.1 to 83 ppm; the C(3′)-signal is downfield shifted by 0.5 ppm whereas the other C-atoms remain almost unaffected. Similar shifts have been reported by *Tanaka & Yahara* [13] for 3-*O*-*β*-sophorosyl dammarane saponins. Thus the structure of **4** is established as hederagenin 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside which is encountered for the first time in nature. Mild hydrolysis of **4** afforded **2**, identified as hederagenin 3-*O*-*β*-D-glucopyranoside. Spectral data, chromatographic behaviour and m.p. of compounds **1** and **3** correspond in all respect to published data of previously reported saponins, respectively to hederagenin-3-*O*-*α*-L-arabinopyranoside [14] and hederagenin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside [14] [15].

Discussion. – Although hederagenin glycosides are widely distributed in various plants [16], it has been possible to isolate a previously unreported saponin with the relatively rare sophorosyl sugar moiety. According to *Hiller* [17], the 1→2 interglycosidic linkage in saponin diglucosides was only found in ginsenosides [13] [18], in calendulosides [19] and in bassiasaponin [20].

The saponins of the leaves of *Hedera helix* L. possess antifungal activity [21] and antibacterial activity against gram-positive and gram-negative bacteria [22]. Their molluscicidal properties are reported here for the first time. The 100% lethal concentrations of **1**–**4** for *Biomphalaria glabrata* snails were around 3, 15, 8 and 12 ppm solutions, respectively (observed after 24 h). It is noteworthy that the saponins containing arabinose (**1** and **3**) exhibit stronger molluscicidal activity than the glucosides **2** and **4**. Structure-activity relationships as well as toxicity studies against plankton are currently in progress. Minor saponins of common ivy berries have not yet been identified.

The present work was initiated during our stay at Columbia University, New York. We thank Prof. *K. Nakanishi* for his kind hospitality and the *Fonds National Suisse de la Recherche Scientifique* for financial support (grant no. 820.425.76). We also thank Prof. *Cl. Favarger*, University of Neuchâtel, for the identification of plant material; Dr. *J.L. Occolowitz*, Lilly Research Laboratories, Indianapolis for FD./MS. measurements; Prof. *O. Sticher*, ETH Zürich for facilities provided during the final stage of the present work; Prof. *K. Hiller*, Humboldt-University, Berlin for useful informations on saponins. We are most grateful to Dr. *H.P. Striebel*, Ciba-Geigy, Basel for providing snails.

Experimental Part

General remarks. – DCCC. separation was achieved on a *Tokyo Rikakikai* apparatus, Model A, equipped with 300 glass tubes. The solvent system was $CHCl_3$/MeOH/H_2O 7:13:8 in which the

mobile phase consisted of the lower layer. The flow rate was 10–15 ml/h. The elutants were collected in 2 ml fractions and monitored by TLC. on silica precoated aluminium sheets *(Merck)* with the lower layer of $CHCl_3$/MeOH/H_2O 65:35:10. Detection with Godin reagent [23]. Liquid chromatography separation was performed on a *Waters* Prep. LC-500 instrument using a refractive index detector. A silica gel cartridge (30×5 cm) with $CHCl_3$/MeOH 17:3 was employed; flow rate 150 ml/min. – ^{13}C-NMR. spectra were recorded at 25.02 MHz on a *Jeol* PS-100 instrument with pyridine-d_5 as solvent, TMS internal standard. FD./MS. were obtained on a *Varian*-Mat Model 731 mass spectrometer using carbon dendrite emitters; emitter current 19–21 mA.

Isolation and identification. – The fresh berries collected in Tüscherz BE, Switzerland, were treated as indicated under Results (see above). In order to get enough sample for the prep. LC. separation of **2** and **3**, the whole isolation procedure was repeated a second time, yielded 500 mg of a mixture, which finally afforded 140 mg of **2** and 205 mg of **3**. Acid hydrolysis (HCl 4N, 6 h) of **1**–**4** gave the same aglycone identified as hederagenin by ^{1}H- and ^{13}C-NMR. [10] and by MS. of its methyl ester [9].

Methylation was achieved with diazomethane in Et_2O/MeOH 3:1. TLC. of the sugars obtained from the saponin hydrolysates were made on silica gel with AcOEt/H_2O/MeOH/AcOH 65:15:15:20, detection with *p*-anisidine phthalate. Mild hydrolysis of **3** and **4** (HCl 0.5N, 2 h) afforded a mixture of hederagenin, **1** and **3** and a mixture of hederagenin **2** and **4**, respectively. Compound **4**: m.p. 334–336°, Rf 0.40. For saponins **1**–**3**, see [16].

Bioassays were made with snails of the species *Biomphalaria glabrata* reared in aquaria with a continuous circulation of water through an *Eheim* Filter System; water temperature 24°. Snails of uniform sizes were used (average diameter of the shell 9 mm). The tests were carried out by placing two snails in a deionized water solution of known concentration. At several time intervals, the snails were placed on a *Petri* dish, light was shone from the bottom, and the heart-beat was checked by a microscope [24]. In order to dissolve completely the weakly water-soluble saponins **1** and **2**, solutions were placed in an ultra-sonic bath for 1 h at RT. prior to bioassay.

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