VELPETIN – A NEW IRIDOID GLYCOSIDE FROM Nepeta velutina

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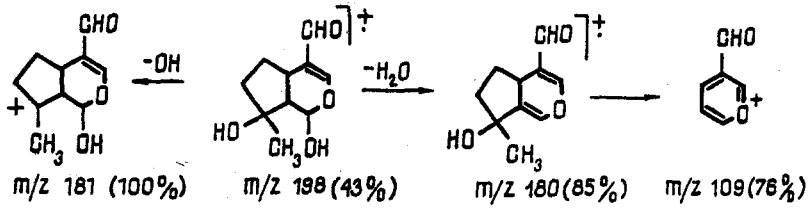
The structure of the new iridoid glycoside velpetin isolated from the herb Nepeta velutina has been established on the basis of spectral characteristics and chemical transformations as 1,5,8,9-tetraepiixoroside.

Four flavenoid compound shave previously been isolated from the herb Nepeta velutina Pojark, family Lamiaceae [1]. At the same time, a white crystalline substance called velpetin was obtained. The present work was devoted to establishing its structure.

Velpetin was assigned to the iridoid glycosides on the basis of the following facts. The UV spectrum of velpetin has an absorption maximum at 249 nm which is typical for iridoids with a conjugated aldehyde group at C-4 [2, 3]. The glycosidic nature of this substance was shown by the NMR spectra of velpetin and of its tetra- and pentaacetates (Tables 1 and 2) and also by the results of acid hydrolysis, which led to the formation of glucose. Under these conditions, the aglycon broke down, but it was obtained by enzymatic hydrolysis and was characterized in the form of an acetate (Table 1).

The electron-impact mass spectrum of velpetin did not contain the molecular ion of the glycoside but id did contain the peak of the ion of the aglycon (m/z 198) and a dominating ion with m/z 181 obviously arising by the elimination of the 8-OH group from the former. The C-1 hdyroxy groups an iridoid aglycon is eliminated mainly in the form of water [4], which, in the case of velpetin, led to the formation of an ion with m/z 180. These ions then lost water, a hydroxyl radical, or CO, forming ions with m/z 163, 162, and 152. Another characteristic ion was that of a peak with m/z 109 [4], shown in the following scheme:



Thus, the mass spectrum and the results of elementary analysis showed the molecular formula $C_{16}H_{24}O_9$ and, according to this, a C_{10}-iridoid glycoside.

The fragmentation under discussion corresponds to the suggested structure (I).

Details of the ^1H NMR spectra of velpetin and its derivatives are given in Table 1. In the spectrum of velpetin taken at 200 MHz by the COSY method an assignment has been made of practically all the iridoid protons. The signal of the proton at C-1 appeared in the form of a doublet with a constant of 3 Hz. The proton at C-9, which appeared in the form of a double doublet at 2.34 ppm, interacted with the first and fifth protons. The signal of the C-3 proton resonated in the form of a singlet, which showed the presence of this proton at a double bond close to an oxygen atom, and also indicated the presence of a substituent at C-4.

Singlet signals at 1.20 and 9.00 ppm clearly showed the presence of a tertiary methyl group and an aldehyde group, respectively. The chemical shift of the methyl group permitted it to be assigned to the C-8 position and, consequently, the aldehyde group to C-4.

The presence of tertiary OH groups at C-8 was shown by the absence of splitting of the signal of the methyl group, and also by the difficulty of its acetylation: on the acetylation of velpetin, together with the full acetate, the tetraacetate, retaining a free 8-OH

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TABLE 1. ^{1}H NMR Spectrum of Velpetin and its Derivatives (δ, ppm; J, Hz; internal standard TMS)

Hydrogen atom	Compound and solvent			
	velpetin	velpetin tetraacetate		acetate of the aglycon.
	D_2O	$CDCl_3$	$CDCl_3$	$CDCl_3$
H-1	5,48 d, 3 Hz	5,26 d, 3Hz	5,76 d 1,5 Hz	6,25 d 4 Hz
H-3	7,35 s	7,12 s	7.10 s	7,10 s
H-5	3,03 m	3,10 m		
2H-6	2,15 m, and 1,38m	1,70 m, 2H		
2H-7	1,63 m	1,70 m, 2H		
H-9	2,34 dd, 3and10 Hz	2,30 dd, 3and10Hz	2,62 dd, 1,5and10 Hz	2,30 dd 4and10 Hz
CH_3	1,20 s	1,30 s	1,48 s	1,33
CHO	9,0 s	9,23 s	9,24 s	9,23 s
H-1′	4,69 d., 7,7 Hz			
H-2′	3,17 dd 7,7and 9 Hz			
H-3′,4′,5′	3,25–3,45			
2H-6′	3,78and 3,60 dd, 12 Hz			
Ac	—	2.04 - 2,12 12H (4 Ac)	1,90–2,11 15 H (5 Ac)	2,08 s

TABLE 2. Comparison of the ^{13}C NMR Characteristics of Velpetin and of Known Compounds (II-IV) (δ in D_2, external standard TMS)

Carbon atom	Velpetin (I) (50.3 MHz)	Ixoroside (I) [7] (22.6 MHz)	1,5,9-Triepide-oxyloganic acid (III) [10] (25.1 MHz)	8-Epideoxyloganic acid (IV) [10] (25.1 MHz).
1	100,7	96,4 d	101,49d	97,06 d
3	164.8	164,2 d	153,57d	153,35 d
4	125,0	125,0 s	113,36s	113,75 s
5	28,8	28,6 d	33,58d	33,12 d
6	28,8	28,6 t	32,09t	31,97 t
7	40,5	40,4 t	33.38t	33,51 t
8	80,4	80,2 s	36,82d	36,55 d
9	51,1	51,1 d	43,89d	43.79 d
10	23.9	23.7 q	17 08q	16,79 q
11	195,8	195,4 d	172,09s	172,14 s
1′	103,4	99,2 d	103,49d	99,55 d
2′	73,9	70,3 d	74,42d	73.91 d
3′	76,5	76,4 d	76,96d	76,91 d
4′	70,3	70 3 d	70,57d	70,74 d
5′	77,3	77,1 d	77,54d	77,42 d
6′	61.5	61.5 t	61.95t	61,94 t

group, was obtained. A comparison of their ^{1}H NMR spectra showed that the signal of the CH_3 group in the 8-acetoxy derivative was shifted downfield by 0.18 ppm in comparison with the corresponding signal of the 8-hydroxy derivative.

The signals of the protons at C-6, 7 in the 1.3-2.1 ppm region showed the presence of a $-CH_2-CH_2-$ chain in a ring.

Attachment of the glucose residue to the OH group at C-1 was confirmed by comparing the chemical shifts of the H-1 proton in the PMR spectra of the aglycon, of the glycoside, and of their acetates. The β-configuration of the glycosidic bond followed from the results of enzymatic hydrolysis and the coupling constant of the anomeric proton of the glucose resin, which was 7.7 Hz.

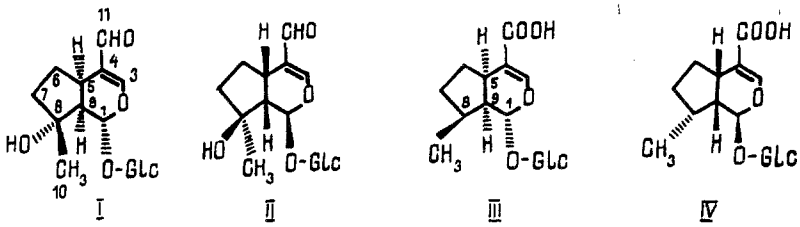
Thus, a combination of spectral characteristics and chemical transformations permitted the suggestion for velpetin of the structure of 4-formyl-8-methyl-3,4-dehydroiridan-1,8-diol-1-O-β-D-glucopyranoside (I). (See scheme on following page.)

The iridoid ixoroside (II) with an analogous structure is described in the literature [6]; however, its physicochemical constants differed considerably from those of velpetin (Table 3), and their acetates also differed (ixoroside Pentaacetate [6]; mp 95-96°C,

TABLE 3. Comparison of Some Constants of Velpetin and the Known Compounds (II-IV)

Compound	mp, °C	$[\alpha]_D$ in MeOH, deg	Chemical shifts of the signals of certain protons (δ ppm)		Maximum on the CD curve in MeOH	Literature
			H-1	H-1'		
I	204—205	+120,3	5,48	4,69	ΔE_{241} +3,68	
II	amorph.	—102,6	5,73	—	—	6
III	105 (decomp.)	+85.1	5,27	4,53	ΔE_{229} +4,4*	10
IV	219—220	—110,1	5,46	4,69	ΔE_{229} —3,7*	10

*The CD curve was taken for the aglycon of the methyl ester.



I II III IV

$[\alpha]_D$ −102.2°). Ixoroside is an amorphous powder, while velpetin is a crystalline substance with a sharp melting point, but their main difference consists in the opposite signs of their specific rotations (see Table 3) which permits them to be regarded as stereoisomers.

Since the stereochemistry of the three asymmetric centers (C-1, C-5, and C-9) was the same in practically all the iridoids identified hitherto [2-7], we assume that velpetin could be the 8-epimer of ixoroside. The configuration of the tertiary OH group at C-8 in iridoid glycosides can be determined by comparing the chemical shifts of the C-1 and C-9 protons in the 8-hydroxy compounds and the corresponding acetoxy compounds [4-6], the shifting effect of a β-acetoxy group at C-8 amounting to 0.2-0.4 ppm, while an α-acetoxy group causes no such effect. The shifts of the H-1 and H-9 signals (Δδ, +0.40 and +0.32, respectively) in the PMR spectrum of velpetin pentaacetate as compared with the tetraacetate showed that velpetin was not 8-epiixoroside.

Thus, substances (I) and (II) must be antipodes with respect to the aglycon. In harmony with this conclusion are the ^1H and ^{13}C NMR spectra and the CD curves of velpetin in comparison with those of compounds (II-IV) (see Tables 2 and 3).

1,5,9-Triepideoxyloganic acid (III) and 8-epideoxyloganic acid (IV) [8-10] form the only pair of antipodes among iridoid glycosides. About 400 natural iridoids having the same (1S, 5S, 9R) stereochemistry of the skeletal carbon atoms C-1, C-5, and C-9 have been described in the literature [2-7]. This is obviously explained by the practically complete absence of information on the circular dichroism of the iridoids, including ixoroside (II). The CD curve of velpetin had a positive Cotton effect, while for ixoroside, according to its structure, a negative effect may be expected.

The CD curves are given in the literature for the antipodes of deoxyloganic acid (III) and (IV), these being opposite to one another (see Table 3), while compound (III), like velpetin, has a positive maximum.

In the ^1H NMR spectra of (III) and (IV) the only large difference is observed in the chemical shifts of H-1 and H-1' (see Table 3), the other chemical shifts and constants being almost identical. Moreover, the ^{13}C NMR spectra of these compounds are completely identical with the exception of the signals belonging to C-1 and C-1' (see Table 2). Analogous differences are observed for the ^1H and ^{13}C NMR spectra of velpetin (I) and ixoroside (II) (see Tables 2 and 3).

Thus, velpetin must have the absolute stereostructure of (I), corresponding to 1,5,8,9-tetraepiixoroside.

It must be emphasized that velpetin is the second natural iridoid glycoside with a stereochemistry differing from the usual (1S, 6S, 9R) system. The first compound revealing a (1R, 5R, 8S, 9S) configuration was 1,5,9-triepideoxyloganic acid (III), isolated from

Nepeta cataria, the structure of which has been twice reconsidered in this connection [8-10].

It is interesting that plants of the genus Nepeta contain a series of monoterpene compounds with unusual stereochemistries – for example, stereoisomers of nepetalactone, differing by the C-5, C-9, and C-8 configurations [11-13].

EXPERIMENTAL

Spectral characteristics were obtained on a Varian CH-8 instrument at 70 eV (mass spectra), Specord M40 and Hitachi EPS-3T instruments (UV), and a UR-20 instrument in paraffin oil (IR). ^{1}H NMR spectra were taken on a Varian HA-100D instrument (100 MHz, δ 0 –TMS) while ^{1}H NMR spectrum in D_2O were taken on a Varian Gemini-200 (200 MHz) with assignment of the signals by the COSY method; the ^{13}C NMR spectrum was taken in D_2O on a Gemini-200 instrument at 50.3 MHz with assignment of the signals by the APT method.

CD spectra were taken on a J-20 spectropolarimeter (Japan). Angles of optical rotation were determined on a EPL polarimeter.

Elementary analysis was conducted on a Hewlett–Packard 185B CHN-analyzer. The elementary composition corresponded to the calculated figures. TLC monitoring was effected on Silufol UV 254 plates in the solvent systems chloroform–methanol–water (26:14:3) and chloroform–methanol (6:1), with detection in UV light at 254 nm.

Velpetin (I). White acicular crystals soluble in water. Composition $C_{16}H_{24}O_9 \cdot 0.5H_2O$, mp 204-205°c (from MeOH – chlf, 3:1); $[\alpha]_D^{20}$ 120.3° (0.45; MeOH), +119.5° (0.8; water). λ_{max}^{MeOH} 249 nm, ν_{max} 3640, 3380, 3310, 1645, 1620, 1570 cm^{-1}. CD spectrum (c 0.1; MeOH): ΔE_{241} + 3.68. ^{1}H NMR spectrum – Table 1. ^{13}C NMR spectrum – Table 2. Mass spectrum m/z (int., %): 198(43), 181(100), 180(85), 163(55), 162(45), 152(27), 151(30), 109(76).

Acid Hyrdolysis. Velpetin (I) (10 mg) was heated with 1% HCl at 100°c for 30 min. The aglycon decomposed under these conditions; glucose was detected in the hydrolysate by the PC method.

Enzymatic Hydrolysis of Velpetin. A solution of 50 mg of velpetin in 1 ml of water was heated with an aqueous solution (7 mg in 1.5 ml) of β-glucosidase (Serva) at 38-40°C for 2 h. Glucose was detected in the hydrolysate. A chloroform extract of the aglycon after evaporation was purified on a column of silica gel. This gave a substance with the composition $C_{10}H_{14}O_9$ (M^+ 198).

The aglycon was acetylated by the usual method and the product was purified on silica gel. This gave the monoacetate of the aglycon; its ^{1}H NMR spectrum is given in Table 1.

Acetylation of Velpetin. The acetylation of velpetin (I) (200 mg) with acetic anhydride in pyridine gave a mixture of the penta- and tetraacetates which were separated by column chromatography on silica gel (chlf–hexane).

The tetraacetate of (I) – amorphous substance; its NMR spectrum is given in Table 1.

The pentaacetate of (I) – mp 130-132°C, $[\alpha]_D^{20}$ +139.6° (0.06; chlf); its ^{1}H NMR spectrum is given in Table 1.

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CHEMICAL MODIFICATION OF THE trans,trans-GERMACRANOLIDE STIZOLICIN

SYNTHESIS, MOLECULAR, AND CRYSTAL STRUCTURE OF 6α-ACETOXY-13-METHOXY-1,10; 4,5-DIEPOXY-1,5,7α(H),8,11β(H)-E,E-GERMACR-8,12-OLIDE

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The results of the chemical modification of stizolicin, a sesquiterpene lactone of the trans,trans-germancrane type and, in particular, the epoxidation, saponification, and acetylation of its molecule are discussed. On the basis of the results of an x-ray structural experiment, for 6-acetoxy-13-methoxy-1,10-epoxystizolicin we propose the structure of 6α-acetoxy-13-methoxy-1,10: 4,5-diepoxy-1,5,7α(H),8,11β(H)-E,E-germacr-8,12-olide.

The chemical modification of the molecules of natural compounds is one of the methods for a purposeful change in their biological properties. In this respect, interest is presented by sesquiterpene γ-lactones, which are considered as polyfunctional compounds in chemical transformations [1].

Stizolicin (I), a sesquiterpene lactone of the germacrane type, is known as a compound with a cytotoxic action (LD_{50} = $9.4 \cdot 10^{-1}$ μg/ml and 4.7 μg/ml for cultures of P-388 and KB cells, respectively) and an antitumoral activity in vivo in relation to murine leukemia P-388 (T/C 123% at 16 mg/kg) [2].

In this paper we present the results of the epoxidation saponification, and acetylation reactions of stizolicin (I).

Stizolicin (I) is a trans-trans-germacranolide with the composition $C_{20}H_{26}O_7$, mp 152-153.5°C, $[\alpha]_D^{25}$ −32.4° (C 2.19; ethanol) isolated from Stizolophus balsamita (Lam.) Cass. ex Takht., S. coronopifolius (Lam.) Cass., Centaurea solstitialis L., and Saussurea elongata DC [3, 4].

In order to obtain a hydroxy derivative of stizolicin we performed the saponification of this lactone under various conditions.

As can be seen from the scheme of transformations, the reactions of stizolicin (I) with a 1 M solution of K_2CO_3 in methanol at pH 10-11 gave derivative (II). Its IR spectrum contained absorption bands in the 3450 cm^{-1} region that is characteristic for a hydroxy group, and at 1780 cm^{-1} characteristic for the carbonyl of a γ-lactone. The PMR spectrum lacked the signal of the protons of an ester group but a signal of the protons at C-6 and C-8 appeared in the form of a complex multiplet with its center at 4.15 ppm and so did the signals of the protons of a methoxy group, (singlet at 3.29 ppm (3H)) and of methylene protons at C-13 in the form of two doublets of doublets at 3.52 and 3.82 ppm with $J_{13a,11}$ = 9 Hz, $J_{13a,13b}$ = 2 Hz, $J_{13b,11}$ = 10 Hz for each. See scheme on following page.

To assign the complex multiplet at 4.15 ppm and to establish the structure of the molecule of derivative (II) we performed its acetylation. When (II) was treated with acetic

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