NMR DETERMINATION OF THE STRUCTURE OF PERACETYLATED ICARISIDE B_2 FROM *Veratrum lobelianum* BERNH.

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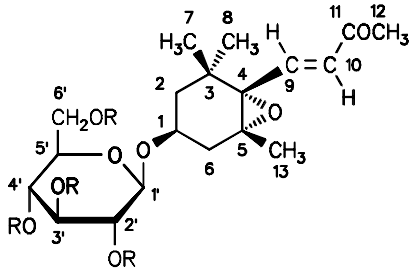
rel(1*S*,4*S*,5*R*)-3,3,5-Trimethyl-4-[(1*E*)-3-oxo-1-butenyl]-4,5-epoxy-cyclohexyl-*O*-β-D-glucopyranoside (*I*) was isolated from the aerial part of *Veratrum lobelianum* BERNH. The structure was derived mainly from detailed analysis of ^{1}H and ^{13}C NMR spectra of its acetylated derivative *II*. Compound *I* has been already reported under the name icariside B_2 in *Epimedium grandiflorum* MORR. var. *thunbergianum* (MIQ.) NAKAI.

In our previous communications[1–6] we described the isolation and structures of glycoalkaloids, waxes, phytosterols, flavonoids and aromatic acids from the aerial part of *Veratrum lobelianum* BERNH.

Our present paper concerns the isolation and identification of *rel*(1*S*,4*S*,5*R*)-3,3,5-trimethyl-4-[(1*E*)-3-oxo-1-butenyl]-4,5-epoxycyclohexyl-*O*-β-D-glucopyranoside (*I*). The UV spectrum of compound *I* exhibits a maximum at 223 nm indicating the presence of an α,β-unsaturated ketone. This is confirmed also by the bands at 1 675 and 1 625 cm^{-1}



I, R = H
II, R = Ac

in the IR spectrum, which revealed another absorptions due to hydroxyl group, methyl and methylene groups. According to its mass spectrum, the compound has a composition $C_{19}H_{30}O_8$ (M^+ 386.1946). The main and characteristic fragmentation consists in the cleavage of the glycoside bond which gives rise to ions of *m/z* 224 ($M - C_6H_{10}O_5$) what is proof, that compound *I* is a glucoside of the genine $C_{13}H_{20}O_3$.

Because of instability of the native substance we have prepared acetyl derivative *II*. Its composition is $C_{27}H_{38}O_{12}$ (M^+ 554.5927); IR spectrum revealed absorptions due to an ester group (1 750, 1 225 cm^{-1}) and α,β-unsaturated ketone. The structure of acetyl derivative *II* was derived from the detailed analysis of ^1H and ^{13}C NMR spectra.

RESULTS AND DISCUSSION

NMR Structure Analysis of Acetyl Derivative II

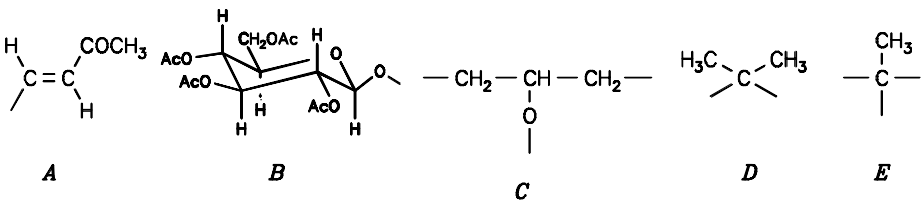
Proton NMR spectrum allowed us to identify the signals of all 38 hydrogen atoms. Proton splitting patterns, decoupling experiments and 2D-COSY spectrum proved the presence of five structural fragments *A* to *E*, which were complemented by carbon-13 NMR data. Proton and carbon-13 NMR data are summarized in Table I.

Fragment *A* gives two doublets of the olefinic protons at δ 7.00 and 6.27 with J = 15.6 Hz (indicating their *trans*-configuration) and a characteristic singlet of acetyl group at δ 2.29.

Fragment *B* gives seven one-proton multiplets of CH–O protons and four singlets of acetate methyl groups. The chemical shift values and vicinal coupling constants (8–10 Hz) are fully compatible with the data of methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside.

Five-proton fragment *C* gives the signals at δ 3.83, 2.31, 1.72, 1.67 and 1.38. Proton distribution CH_2–CH–CH_2 follows from the coupling constant values which – together with the chemical shifts – indicate also the character of substituents (quaternary carbons at the end positions and the oxygen atom in the middle).

Three singlets in the upfield part of ^1H NMR spectrum (δ 1.18, 1.17 and 0.96) belong to three tertiary methyl groups. Two of them – at δ 1.17 and 0.96 – are broadened due to small mutual coupling and can be therefore assigned to the geminal dimethyl group of fragment *D*. The third one (δ 1.18) is a tertiary methyl group representing a fragment *E*.



Proton decoupled ^{13}C NMR spectrum showed 25 signals for 27 carbon atoms present due to signal overlap at δ 72.78 and 20.62. The signals were assigned to CH_3, CH_2, CH and C carbon atoms using the "attached proton test" and the signal intensities. Lowfield region (δ 198–132) contains signals of seven sp^2-carbons which could be assigned to one ketone C=O carbon (δ 197.33), four ester C=O carbons (at δ ≈ 170) and two olefinic

TABLE I
Proton and carbon-13 NMR parameters of compound *II* in $CDCl_3$

Proton	δ, ppm	*J*, Hz	Carbon	δ, ppm
H-9	7.00 d	*J*(9,10) = 15.6	C-11	197.33
H-10	6.27 d	*J*(10,9) = 15.6	C=O (OAc)	170.61
H-3′	5.19 dd	*J*(3′,2′) = 9.6; *J*(3′,4′) = 9.4	C=O (OAc)	170.29
H-4′	5.05 dd	*J*(4′,3′) = 9.4; *J*(4′,5′) = 9.9	C=O (OAc)	169.43
H-2′	4.94 dd	*J*(2′,1′) = 8.0; *J*(2′,3′) = 9.6	C=O (OAc)	169.18
H-1′	4.54 d	*J*(1′,2′) = 8.0	C-9	141.97
H-6′a	4.23 dd	*J*(6′a,5′) = 5.4; *J*(6′a,6′b) = 12.2	C-10	132.78
H-6′b	4.12 dd	*J*(6′b,5′) = 2.4; *J*(6′b,6′a) = 12.2	C-1′	99.90
H-1	3.83 m	*J*(1,2ax) = 9.9; *J*1,2eq) = 3.5;	C-1 + C3′	72.78
		J(1,6ax) = 8.2; *J*(1,6eq) = 5.1	C-5′	71.77
H-5′	3.68 ddd	*J*(5′,4′) = 9.9; *J*(5′,6′a) = 5.4; *J*(5′,6′b) = 2.4	C-2′	71.43
H-6eq	2.31 ddd	*J*(6eq,6ax) = 14.5; *J*(6eq,1) = 5.1;	C-4	69.75[a]
		J(6eq,2eq) = 1.5	C-4′	68.51
CH_3-12	2.29 s		C-5	66.66[a]
OAc	2.01 s		C-6′	62.16
OAc	2.03 s		C-3	43.89
OAc	2.05 s		C-6	37.46[b]
OAc	2.08 s		C-2	34.93[b]
H-2eq	1.72 dd	*J*(2eq,2ax) = 13.3; *J*(2eq,1) = 3.5;	C-13	28.96[c]
		J(2eq,6eq) = 1.5	C-7	28.18[c]
H-6ax	1.67 dd	*J*(6ax,6eq) = 14.5; *J*(6ax,1) = 8.2	C-8	25.13[c]
H-2ax	1.38 dd	*J*(2ax,2eq) = 13.3; *J*(2ax,1) = 9.9	CH_3 (OAc)	20.76
CH_3-13	1.18 s		CH_3 (AOc)	20.69
CH_3-7	1.17 s		2 × CH_3 (2 × OAc)	20.62
CH_3-8	0.96 s		C-12	19.96

[a,b,c] The signals with same symbols were assigned tentatively and may be mutually interchanged.

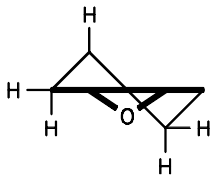
–CH= carbon atoms (signals at δ 141.97 and 132.78). The middle region (δ 100–60) shows signals of nine carbon atoms bonded to oxygen atoms. Six of them can be assigned to the carbohydrate unit by comparison with the literature data for methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside[7]. Three residual CH–O carbons (signals at δ 72.78, 69.75 and 66.66) must be located in a bicyclic system of a genin part of molecule. Upfield region (δ 45–20) contains the signals of the eleven carbon atoms. Five of them (at δ ≈ 20) can easily be identified as methyl carbon atoms of acetyl groups, three further signals (δ 25–29) belong to tertiary methyl groups and finally there are two signals (δ 37.46 and 34.93) of CH_2 carbon atoms and one signal (δ 43.89) of quaternary carbon atom.

The final structure was derived mainly from detailed analysis of interproton coupling constants in the relation to molecular models of possible structures and from proton NOE data. Molecular formula $C_{27}H_{38}O_{12}$ and the identified structure fragments indicate that genin part of the molecule (containing the fragments *A*, *C*, *D*, and *E*) must form a bicyclic system of six carbon atoms and etheric oxygen with acetylated β-D-glucopyranose, 3-oxo-but-1-enyl, geminal dimethyl and tertiary methyl group as substituents. In general the bicyclic system (containing already identified fragment *C*) may consist of either two five-membered rings, or six- and four-membered ring, and/or six- and three-membered ring. The observed values of vicinal coupling constants of –CH– proton in $–CH_2–CH–CH_2–$ fragment (9.9, 3.5 Hz and 8.2, 5.1 Hz) clearly indicate the presence of axial proton in the vicinity of axial, equatorial and pseudoaxial, pseudoequatorial protons in a cyclohexane ring with the 1,2-epoxy group (fragment *F*). It should accept a twist-chair form with acetylated β-D-glucosyl residue in the equatorial position. Four-bond coupling 1.5 Hz between equatorial and pseudoequatorial proton (δ 1.72 and 2.31) is characteristic for similar cyclic systems.

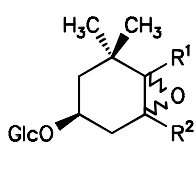
In the next step we had to determine the position of two substituents – 3-oxo-1-butenyl and methyl group – on the epoxide carbon atoms. For distinguishing between two alternative structures *G*, *H* we have measured 2D-ROESY spectrum of compound *II*. The observation of the ROESY crosspeaks between both protons of one methylene group and methyl substituent on one side and between one of geminal methyl groups and the olefinic proton of 3-oxo-1-butenyl on the other side is consistent with structure *H* only.

Finally we had to establish a relative configuration of tetraacetylated β-D-glucosyl and epoxide ring. The absence of proper interproton coupling constants and geometry properties of epoxide (small differences in orientation of CH_3 and $CH=CH–COCH_3$ substituents accompanying the change of epoxide configuration) make the determination not straightforward. The structures *K* and *L* show the isomers with *cis*- and *trans*-configuration of tetraacetyl β-D-glucosyl and epoxide ring, respectively. The observed crosspeaks in the ROESY spectrum of compound *II* better fit the structure *L* (ROESY peaks between H-1 and olefinic protons H-9, H-10 which could be expected in structure *K* where given protons are *cis*-oriented had not been observed). Therefore we could con-

clude that the acetylated derivative is represented by formula *II* and the structure of natural hydroxy compound is given by formula *I* (both representing the relative configuration).

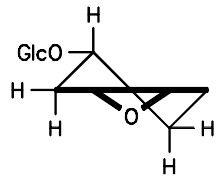


F

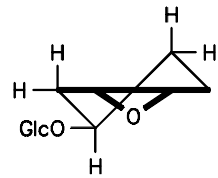


G, $R^1 = CH_3$, $R^2 = -CH{=}CH{-}COCH_3$

H, $R^1 = -CH{=}CH{-}COCH_3$, $R^2 = CH_3$



K



L

Structure *I* was reported already by Miyase et al.[8] under the name icariside B_2 for compound isolated from *Epimedium grandiflorum* var. *thunbergianum* (MIQ.) NAKAI. Some physical and spectral data of compound *I* (m.p., UV) agree with those reported[8]. NMR data could not be compared (acetylated derivative *II* is not described in ref.[8]).

EXPERIMENTAL

Apparatus

The melting points were determined on a Kofler micro hot-stage. Optical rotations of methanolic solutions were measured with the respective Polamat A (Zeiss, Jena). UV spectra were taken on a UV-VIS instrument (Zeiss, Jena) in methanol; IR spectra on a Perkin–Elmer 580 spectrophotometer by the KBr technique (wavenumbers in cm^{-1}). Mass spectra were obtained with an AEI MS 902 instrument (electron energy 70 eV). NMR spectra of compound *II* were measured on Varian UNITY-500 FT NMR spectrometer (^1H at 500 MHz; ^{13}C at 125.7 MHz) in $CDCl_3$ solution with tetramethylsilane as an internal reference in ^1H and ^{13}C NMR spectra. Silica gel No. 5 (Silpearl), modified according to Pitra et al.[9], silica gel G (according to Stahl, Merck) and Silufol UV_{254} and UV_{366} sheets were employed for column and thin-layer chromatography, respectively.

Extraction and Isolation of the Compound *I*

The air-dried and ground drug (20 kg) was extracted stepwise with benzene and ethanol. The ethanolic extract was concentrated under diminished pressure to 25 l at a temperature not exceeding 45 °C. The concentrated ethanolic extract was processed according to ref.[5].

Fractions 33–45 afforded 18 mg of crystalline *I*, m.p. 171–172 °C, $[\alpha]_{578}$ –136.8°, R_F 0.54 (chloroform–methanol 7 : 3). UV spectrum, λ_{max} nm (log ε): 233 (4.46). IR spectrum: 3 400, 2 980, 2 940, 2 910, 1 675, 1 640, 1 625, 1 480, 1 430, 1 385, 1 325, 1 295, 1 265, 1 242, 1 165, 1 105, 1 080, 1 040, 985, 975, 955, 945, 910. Mass spectrum, *m/z*: M^+ 386.1943 for $C_{19}H_{30}O_8$ (calculated 386.2352), 224 ($M - C_6H_{10}O_5$).

Acetylation with acetanhydride in pyridine (room temperature; 24 h) afforded tetraacetyl derivative *II*, m.p. 154–155 °C, $[\alpha]_{578}$ –90.9°. UV spectrum, λ_{max} nm (log ε): 232 (4.42). IR spectrum: 2 965, 2 940, 2 870, 1 750, 1 670, 1 645, 1 630, 1 445, 1 430, 1 386, 1 365, 1 330, 1 250, 1 225, 1 175, 1 145, 1 125, 1 085, 990, 960, 940. Mass spectrum, *m/z*: 554.5927 for $C_{27}H_{38}O_{12}$ (calculated 554.5927).

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