THE STRUCTURE OF HYDROXYISONOBILIN — A CYTOSTATICALLY ACTIVE SESQUITERPENIC LACTONE FROM THE LEAVES OF *Anthemis nobilis* L.

Z.SAMEK[a], M.HOLUB[a], H.GRABARCZYK[b], B.DROŻDŻ[b] and V.HEROUT[a]

[a] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6*
[b] *Biological-Pharmaceutical Institute, Medical Academy, Poznań, Poland*

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From the leaves of *Anthemis nobilis* L. hydroxyisonobilin *V*, a sesquiterpenic lactone, was isolated. Its structure was derived on the basis of physical methods, mainly ^{1}H-NMR spectroscopy.

Some time ago the isolation of nobilin (*I*), a sesquiterpenic lactone from the flowers of *Anthemis nobilis* L. (family *Compositae*, tribe *Anthemideae*), has been described and its structure proposed[1]. In later papers[2,3] the originally proposed structure was corrected to formula *I*. Simultaneously the isolation of dehydronobilin (*II*), 1,10-epoxynobilin (*III*) and 3-epinobilin[3] (*IV*) was described, which are present in relatively small amounts in the lactonic fractions of the flowers of *A. nobilis*. Further the lactone fraction obtained from the leaves of *A. nobilis* was also studied and a sesquiterpenic lactone[4] of m.p. 144–146°C, $[\alpha]_D$ +35·4°, and the composition $C_{20}H_{26}O_6$ was isolated from it as the main component, named hydroxyisonobilin (*V*). Its IR spectrum shows absorption bands at 1760 and 1158 cm^{-1} (α,β-unsaturated exomethylene γ-lactone), 1714 cm^{-1} (ester group with a conjugated double bond), 1652 cm^{-1} (double bond), and 3475 and 3605 cm^{-1} (hydroxyl group). The mass spectrum of compound *V* had its molecular peak at *m/e* 362 and the fragments 262 (M – 100), 244 (M – 100–18), 226 (M – 100–18–18), 83 ($C_4H_7.CO^+$) and 55 ($C_4H_7^+$). The hydroxyl groups of hydroxyisonobilin (*V*) do not belong to a vicinal diol, as shown by its inertness towards oxidation with periodic acid.

The structure of hydroxyisonobilin (*V*) followed directly from analysis of its 100 MHz ^{1}H-NMR spectra and frequency swept decoupling experiments. The assignment of the characteristic protons of the spectrum is the following (in deuteriochloroform with addition of polysol, tetramethylsilane as internal standard, first order data,

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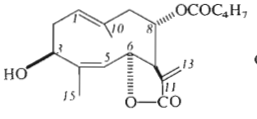
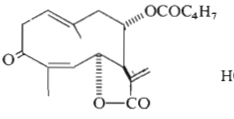
chemical shifts in ppm): H_1: 4·06 dd ($J_1 = 4$; $J_2 = 10$); H_3: 4·48 dd ($J_1 = 3·5$; $J_2 = 4$); H_5: 5·27 dq ($J_{5,6} = 10$); H_6: 6·28 dd ($J_{6,7} = 3·3$; $J_{6,5} = 10$); H_7: 3·11; H_8: 5·11 td ($J_{8,9} = 4$, $J_{8,7} = J_{8,9'} = 10$); H_9: 2·98 dd ($J_{9,8} = 4$; $J_{9,9'} = 14$); $H_{9'}$: 2·44 dd ($J_{9',8} = 10$; $J_{9',9} = 14$); H_{13}: 6·27 ($J_{13,7} = 2·2$; $J_{13,13'} = 0·5$); $H_{13'}$: 5·68 ($J_{13',7} = 1·8$; $J_{13',13} = 0·5$); H_{14}: 5·53 bs; $H_{14'}$: 5·39 bs; H_{15}: 1·78 bd ($J_{15,5} = 1·4$); β-H (angelyl). 6·08 qq. The observed distributions of the chemical shifts and the values of the vicinal and long-range couplings of protons on the fragment $C_{(3)}$—$C_{(4)}(C_{(15)})$—$C_{(5)}$—$C_{(6)}$—$C_{(7)}(C_{11})$—$C_{(13)})$—$C_{(8)}$ are practically identical as in the case of nobilin (*I*) and related native sesquiterpenic lactones[3], which permitted the assumption that in the case of hydroxyisonobilin the relative configuration of the mentioned fragment will also be the same. The acylation experiments obtained by *in situ* acylation of both hydroxyl groups with trichloroacetyl isocyanate[5,6] were also informative ($\Delta^{(i,k)}\delta H_{(j)}(R) = \delta H_{(j)}$ ($C_{(i,k)}$—OH) $- \delta H_{(j)}(C_{(i,k)}$—OR); $(i,k) = 1$ and 3; R = $CONHCOCCl_3$). The shifts $\Delta^{(1,3)}\delta H_1 = -1·28$ and $\Delta^{(1,3)}\delta H_3 = -1·05$ ppm are consistent with the α-shifts with the secondary alcoholic groups (NH: 9·08 and 9·03 ppm) and simultaneously $\Delta^{(1,3)}\delta H_5 = -0·17$, $\Delta^{(1,3)}\delta H_{15} = -0·19$, $\Delta^{(1,3)}\delta H_{14} = -0·29$, $\Delta^{(1,3)}\delta H_{14'} = -0·36$ are consistent with the γ-shifts for vicinal alcohols of this type and also in agreement with the position of both hydroxyl groups and double bonds (for the types OH—C—$C(CH_3)$=CH— see ref.[3]; for the types HO—C—C(R)=CH_2 see ref.[7,8]). The shifts $\Delta^{(1,3)}\delta H_6 = +0·53$, $\Delta^{(1,3)}\delta H_8 = -0·03$, $\Delta^{(1,3)}\delta H_{13'} = -0·07$ entirely correspond to the same shifts of $\Delta^{(3)}\delta H_{(j)}$ in nobilin (*I*) and in related native lactones[3], in agreement with the supposed stereochemistry of the molecule. Hence the structure *V* follows for hydroxyisonobilin.

Hydroxyisonobilin (*V*) is related to germacranolides with a *trans* double bond between $C_{(1)}$ and $C_{(10)}$ and with a *cis* double bond between $C_{(4)}$ and $C_{(5)}$, *i.e.* to heliangolides[9], as for example to sesquiterpenic lactones such as nobilin (*I*), 3-dehydronobilin (*II*), 1,10-epoxynobilin (*III*) and 3-epinobilin (*IV*), which were isolated from the flowers of *A. nobilis*[1,3]. The structure of hydroxyisonobilin differs to a certain extent from that of the mentioned series in the localisation of the double bond between $C_{(10)}$ and $C_{(14)}$ and the hydroxyl group in $C_{(1)}$. Up to now eight germacranolides with an exomethylene double bond between $C_{(10)}$ and $C_{(14)}$ have been described, namely artemorin[10] (*VI*), verlotorin[10] (*VII*), anhydroverlotorin[10] (*VIII*), ridentin[11] (*IX*), dihydroridentin[11] (*X*), artevasin[12,13] (*XI*), chrysanolide[14] (*XII*), and tamirin[15](*XIII*). All these lactones were isolated from the species of the *Anthemideae* tribe (genera *Artemisia*, *Pyrethrum* and *Tanacetum*), among which *Anthemis nobilis* L. also belongs. But hydroxyisonobilin (*V*) represents the first described representative of these substances, in which the endocyclic double bond between $C_{(4)}$ and $C_{(5)}$ is *cis*.

Hydroxyisonobilin (*V*) displays a considerable *in vitro* activity against human tumor cells of carcinoma cervicis uteri (HeLa) and nasopharynx carcinoma[4] (KB).



I



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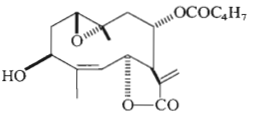

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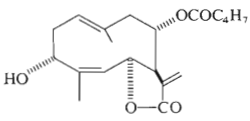
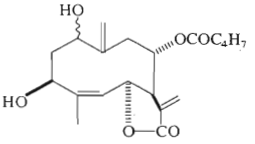
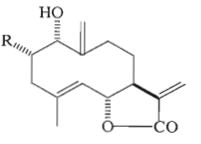


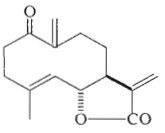
IV



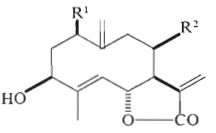
V



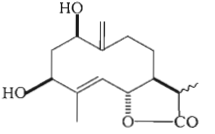
VI: R = H
VII: R = OH



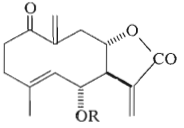
VIII



IX: R^1 = OH; R^2 = H
XI: R^1 = H; R^2 = OH



X



XII: R = $COCH_3$
XIII: R = H

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. The IR spectrum was measured in chloroform on a Zeiss UR-10 (Jena) spectrophotometer. The ^{1}H-NMR spectra were measured on a Varian HA 100 apparatus. The mass spectrum was measured on an AEI MS 902 instrument. Optical rotation was determined with an objective polarimeter in methanol. Circular dichroism was measured on a Roussel Jouan Dichrograph CD 185 in methanol.

Hydroxyisonobilin (*V*)

Hydroxyisonobilin (*V*) was obtained in the manner described[4] and had m.p. 144—146°C (methanol, ether) and $[\alpha]_D^{20}$ +35·4° (*c* 0·35). IR spectrum (in cm^{-1}): 1760, 1158 (exomethylene-γ-lactone), 1714 (α,β-conjugated ester), 1652 (double bond), 3475 and 3605 (hydroxyl). Mass spectrum: 362 (M), 262 (M — 100), 244 (M — 100—18), 226 (M — 100—18—18), 83 ($C_4H_7.CO^+$), 55 ($C_4H_7^+$). CD: $\Delta\varepsilon_{215}$ −6·74; $\Delta\varepsilon_{233}$ ±0; $\Delta\varepsilon_{243}$ +1·69; $\Delta\varepsilon_{300}$ ±0. For $C_{20}H_{26}O_6$ (362·4) calculated: 66·28% C, 7·23% H, 0·56% H act. (2); found: 66·02% C, 7·42% H, 0·40% H act.

The elemental analysis was carried out in the analytical department of our institute, the infrared spectra were measured by Mr P. Formánek and the CD curve by Dr S. Vašíčková. The mass spectrum was measured and interpreted by Dr L. Dolejš. We express our sincere thanks to all those mentioned.

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