XERANTHOLIDE — A NEW CYTOTOXICALLY ACTIVE SESQUITERPENIC LACTONE FROM *Xeranthemum cylindraceum* SIBTH. *et* SMITH*

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From the above-ground part of the plant species *Xeranthemum cylindraceum* SIBTH *et* SMITH. an as yet undescribed sesquiterpenic lactone has been isolated. On the basis of physical methods, mainly ^{1}H-NMR spectroscopy, its partial stereostructure represented by formula *VIII* and the name xerantholide have been proposed. Chemical correlation with mikanocryptin has also been carried out. This substance displayed a considerable cytostatic activity *in vitro* against the HeLa and KB type tumor cells.

In connection with a study of sesquiterpenic lactones from species of the *Compositae* family we also investigated the plant species *Xeranthemum cylindraceum* SIBTH. *et* SMITH (synonymum *X. inapertum duct. non* MILL., *X. sesamoides* GAY., *X. anuum* SCOP. *non* L., *X. foetidum* (CASS.) MOENCH) from the tribe *Cynareae*. From the overground part of this species we isolated a lactone fraction using the method described in ref.[1], which was chromatographed twice on a silica gel column to yield compound *I*, m.p. 175–177°C, $[\alpha]_D^{20}$ +239°, of the composition $C_{15}H_{18}O_3$ (mass: 246), which we have named xerantholide. The IR spectrum of xerantholide (*I*) contained absorption bands at 1770 and 1158 cm^{-1} corresponding to an exomethylene--γ-lactone arrangement, and bands at 1695 and 1638 cm^{-1} probably due to the presence of a keto group in a five-membered cycle conjugated with a double bond. In accordance with this fact the UV spectrum of xerantholide had an absorption maximum at 239 nm (log ε 4·10).

The ^{1}H-NMR spectrum of compound *I* (100 MHz, $CDCl_3$) contained characteristic signals of two exomethylene protons of the α,β-unsaturated γ-lactone ring at 6·29 ppm (d, J = 3·4) and 5·60 ppm (d, J = 2·9), one proton of the RO—CH type at 4·13 ppm (ddd, J_1 = 3·5, J_2 = 9, J_3 = 11·5), and three protons of the methyl group on a tetrasubstituted double bond at 1·77 ppm (m). From the elemental com-

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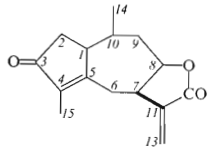
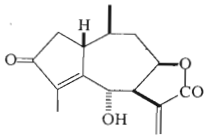
position, the IR, UV and ^{1}H-NMR spectrum the following conclusions could be drawn: 1) the substance is a keto lactone with an exomethylenic γ-lactone ring annelated with a 0,3,5-bicyclodecane skeleton containing an α,β-unsaturated keto group in a 5-membered ring; the double bond conjugated with the carbonyl carries a tertiary methyl group which – according to its chemical shift – must be in α-position to the carbonyl. 2) The presence of two skeletal methyl groups, of which one is secondary (sp^3-type) and the other tertiary (sp^2-type), corresponding to two C_1 side chains of the secondary type in a saturated skeleton (C_1)—CH(C,C), and a C_3-unit in the lactone ring, corresponding to an isopropyl side chain of the secondary type in a saturated skeleton (C_3)—CH(C,C), indicated the probability that the substance is a standard sesquiterpenic lactone of guaiane type with the usual distribution of side chains at $C_{(4)}$, $C_{(10)}$ and $C_{(7)}$ (using standard numbering). The signal of the proton RO—CH is caused by the proton bound to the carbon atom at which the lactone ring is closed, while from the observed multiplicity, which indicated the presence of coupling with three different protons (confirmed by DR experiments), it followed that it is carbon $C_{(8)}$.

From all these aspects it followed that xerantholide has the structure shown by formula *I*. According to structural elements in structure *I* a structural relatedness could be postulated with some already described guaian-8,12-olides which contain the same cyclopentene ring as for example geigerin (*II*) and its 1,10,11-stereoisomeric 6-deoxy derivatives[2–4] or mikanocryptin (*III*) (ref.[5]) and its 1,10,11-stereoisomeric 6-deoxy derivatives. Both these types of substances differ substantially in the stereochemistry of the lactone ring; the geigerin series are *cis*-lactones, while the mikanocryptin series are *trans*-lactones. From the values of the vicinal coupling $J_{7,8} = 9$ Hz (identified by DR experiments) and from the long-range allylic interactions $J_{7,13} = 3{\cdot}4$ and $J_{7,13} = 2{\cdot}9$ in the ^{1}H-NMR spectrum of xerantholide it follows that it is evidently a trans lactone (lactone rule: $J_{7,13}$ (*trans*-lactone) $\geqq 3 \geqq J_{7,13}$ (*cis*-lactone))[7], and hence a substance related with mikanocryptin.

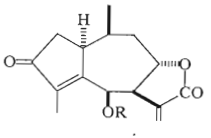
The stereochemistry of the lactone ring in mikanocryptin has been recently confirmed by X-ray analysis[8]. The validity of the mentioned lactone rule[7] for the implications of the stereochemistry of the lactone ring from $J_{7,13}$ was subjected to a detailed revision the results of which will be published elsewhere. As already mentioned[9] the validity is dependent on a number of conformational factors. Of fundamental importance is both the value of the angle $\Phi_R = \sphericalangle C_{(7\pm2)}—C_{(7\pm1)}—C_{(7)}—C_{(7\mp1)}$ and the orientation of the $C_{(7)}—C_{(11)}$ bond. In the range of conformations with $\Phi_R < 120$ and an equatorial $C_{(7)}—C_{(11)}$ bond the limitation of the ranges of the allylic angle $\Phi_{7,13} = \Phi_{7,11}$ may be expected, adequate to the postulated rule, *i.e.* $0 \leqq \Phi_{7,13} \leqq 60$ for *cis*-lactone and $60 \leqq \Phi_{7,13} \leqq 120$ for *trans*-lactone, and hence a good correlation between $\Phi(H_7, H_{(7\pm1)})$ and $\Phi_{7,13}$ and these values and $J_{7,13}$ may also be expected. Such conditions are usually well fulfilled for the annelation of the lactone ring to small homocycles, for example with $n = 5, 6, 7$, such as occur, for example in santanolides or guaianolides, and less so for $n = 10$, such as occur for example in heliangolides[9].



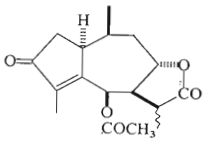
I



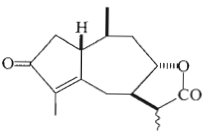
II



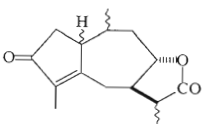
III, R = H
IV, R = $COCH_3$



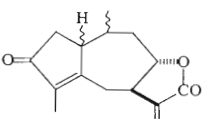
V



VI



VIIa, b



VIII

This relatedness is demonstrated in Table I on some characteristic signals of the ^{1}H-NMR spectra of xerantholide *I*, acetylmikanocryptin (*IV*) (ref.[5]), 11,13-dihydroacetylmicanocryptin (*V*) (ref.[5]) and 6-deoxy-11,13-dihydromikanocryptin (*VI*) (ref.[5]). We also carried out an attempt at direct correlation of xerantholide with mikanocryptin *via* the 6-deoxy derivative *VI* which could potentially be identical with 11,13-dihydroxerantholide.

TABLE I

Comparison of Characteristic Parameters of ^{1}H-NMR Spectra of Xerantholide (*I*), 11,13-Dihydroxerantholide (*VIIa*, *VIIb*) with 6-Acetyl-mikanocryptin (*IV*), 6-Acetyl-11,13-dihydromikanocryptin (*V*) and with 6-Deoxy-11,13-mikanocryptin (*VI*)

Substance	H_1	H_2	$H_{2'}$	H_7	H_8	H_9	$H_{9'}$	H_{13}	H_{14}	H_{15}
I[b]	3·20	2·67 dd (7; 19)	2·12 dd (2·19)	3·05	4·13 ddd (3·5; 9; 11·5)	2·56 m ($J_{9,8}$ = 3·5)	1·92 m ($J_{9',8}$ = 12)	6·29 d (3·4) 5·60 d (2·9)	0·78 d (7·2)	1·77 m
IV[c]	3·9	2·70 dd (19·4; 6·6)	2·19 (2·0; 19·4)	3·23 $J_{7,8}$ = 10	4·64 ddd (12·4; 10; 3·7)	2·45 dt (12·8; 3·7; 3·4)	1·95 (12·8; 12·4; 4)	6·35 d (3·5) 5·86 d (3·1)	0·86 d (7·5)	1·69 dd (1; 0·9)
V[d]	3·18	—	—	2·62	4·98 ddd (11·5; 10; 3·7)	2·5	1·9	1·27 d (7)	0·96 d (7·5)	1·75 d (1·7)
VI[d]	3·17	—	—	2·62	4·33 (12; 10; 3·7)	—	1·95 m	1·29 d (6·7)	0·73 d (6·8)	1·70 dd (1; 0·9)
VIIa[b,e]	—	—	—	—	4·15 ddd (3·5; 9; 11·5) [4·18]	—	—	1·28 d (6·6) [1·30]	0·74 d (7·1) [0·73 d]	1·72 m [1·70]

[a] All compounds measured in deuteriochloroform; internal standard tetramethylsilane; data from first-order analysis; multiplicity given (if clear) as usual (splittings in parentheses); [b] from 100 MHz spectra (Varian-HA-100); [c] from 270 MHz spectra; [d] from 90 MHz spectra; [e] numbers in square parentheses belong to the chemical shifts of protons of *VIIb* (These data are derived from a 60 MHz spectrum).

Hydrogenation of xerantholide, carried out in the same manner as in the case of mikanocryptin, afforded substances *VIIa* and *VIIb*, having close R_F values, which in their ^{1}H-NMR spectra displayed practically identical chemical shifts of signals of $H_{(8)}$, $H_{(13)}$, $H_{(14)}$ and $H_{(15)}$. The signals of these protons were also practically identical with the corresponding signals of *VI*. Significant differences are observed for $H_{(8)}$ and $J_{10,14}$ only. Unfortunately substance *VI* was not at our disposal. We had its spectra only (of which the ^{1}H-NMR spectrum was on a 90 MHz instrument), so that it was impossible to compare the spectra at identical conditions or to evaluate other criteria. Similarly we have been unable to carry out the preparation of dihydroxerantholides at an adequate scale so far, owing to a lack of starting substance. For these reasons we could not solve unambiguously the question of the identity of substance *VI* with some of the 11,13-dihydroxerantholides, or the stereochemistry of the centres $C_{(1)}$ and $C_{(10)}$. In view of the similarity of the characteristic signals of the three isomers described it will be necessary to perform a more detailed comparison of other parts of the spectra, especially of the signals of $H_{(1)}$ and $H_{(10)}$, which were inaccessible under the given conditions. It was also impossible to make unambiguous conclusions from the study of the CE-effects of both chromophores present. A potential complication in the case of these substances could be the epimerization of the centre $C_{(1)}$, as has already been indicated by Herz and co-workers[5]. At this stage we propose for xerantholide the partial stereostructure *VIII*. The question of the centres $C_{(1)}$ and $C_{(10)}$, as well as the absolute configuration remains further a subject of our studies and it will be discussed in a broader context in one of subsequent communications.

The cytotoxic activity was determined, using the method of tissue cultures, on human tumor cells KB (*nasopharynx carcinoma*) and on HeLa cells (*carcinoma cervicis uteri*) according to Geran and co-workers[10]* with some modifications[11–13]. The comparison of the given results obtained with substances *I*, *IX* and *X* (Table II) and the comparison with the data on other substances[11,12,14] leads to the conclusions that the tested substance displays cytostatic activity. The values of a two-step determination of ED_{50} on HeLa and KB cells are lower than the required value, 4 μ/ml (ref.[10]), and so qualify xerantolide (*I*) for further testing *in vivo*.

EXPERIMENTAL

The melting points were determined on a Kofler block and they were not corrected. The IR spectra were measured in chloroform on a Zeiss UR-10 (Jena) spectrophotometer. The ^{1}H-NMR spectra were measured on a Varian HA-100 instrument. The mass spectra were measured on an AEI MS 902 apparatus. The ultraviolet spectrum was measured in methanol on a Specord

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UV VIS. Optical rotation was determined on an objective polarimeter in methanol. Circular dichroism was measured with a Roussel Jouan Dichrographe CD 185 in methanol.

Isolation of Xerantholide (*I*)

From the fresh overground part of *X. cylindraceum* (6·0 kg) a lactone fraction (4·2 g) was obtained in the described manner[1] which was chromatographed on 200 g of silica gel under elution with chloroform and then with a mixture of chloroform with increasing amount of acetone (10%, 20%, *etc.*, up to 50%). The course of chromatography was controlled by thin layer chromatography on silica gel. From the first fractions, which had a tendency to crystallize and which according to thin-layer chromatography contained a single substance only, compound *I* (500 mg) was obtained by chromatography, m.p. 175—177°C and $[\alpha]_D^{20}$ +239·6° (c = 0·484). IR spectrum (in cm^{-1}): 1 770 and 1 158 (exomethylene-γ-lactone), 1 695 and 1 638 (conjugated ketone in a five-membered ring). UV: λ_{max} 239 nm (log ε 4·10). Mass spectrum (*m/e*): 246 (M). CD spectrum (λ, nm, $\Delta\varepsilon$): 209 (±0); 238 (+9·30); 281 (±0); 317 (−0·68). For $C_{15}H_{18}O_3$ (246·3) calculated: 73·14% C, 7·37% H; found: 72·85% C, 7·50% H.

TABLE II

Results of the Determination of Cytostatic Activity of Xerantholide (*I*) in Tissue Cultures of Human Neoplastic Cells

Substance	Solvent	ED_{50}[a] HeLa[b] µg/ml	range	mol/l	KB[c] µg/ml	range	mol/l
I	DMSO[d]	1·45	0·8—2·5	$5{\cdot}9 \cdot 10^{-6}$	1·503	0·43—3·90	$6{\cdot}1 \cdot 10^{-6}$
IX[e]	H_2O	0·89	0·2—3·5	—	—	—	—
X[f]	H_2O	$8 \cdot 10^{-4}$	6 to $8 \cdot 10^{-4}$	—	—	—	—

[a] Concentration of substance inhibiting by 50% protein biosynthesis of the cell population. The numbers indicate arithmetical mean of three measurements carried out under the following conditions: time of exposure 72 h; nutritive medium pH 7·2. ED_{50} was determined by computer analysis[13]. [b] The corresponding substance was tested in three concentrations, using the difference in the dose 1 log. [c] In the second step the substance was tested in five concentrations, using a difference in the dose 0·3 log. Minimum effective dose ED_0 = 0·406 and correlation coefficient R^2 = 90·3 (ref.[13]). Standard deviation of log ED_{50} did not exceed 0·267. The expected mean value of the substance activity in 95% of cases oscillated within the activity limits of $ED_{50} = A$, from A/3·4 to A × 3·4. — [d] Dimethyl sulfoxide; ED_{50} 500 > µg/ml. [e] Cytosine arabinoside (Cytarabin Upjohn) as the first standard. [f] Actinomycin D (Dactinomycin Merck, Sharp and Dohme) as the second standard.

Dihydroxerantholides *VIIa* and *VIIb*

Xerantholide (*I*, 17 mg) was dissolved in 2 ml of benzene, 4 mg of tris(triphenylphosphine)rhodium (*I*) chloride (Fluka) were added and the mixture was saturated with hydrogen at 24°C and 743 Torr. After 3 days the hydrogenation was stopped, the solvent distilled off in a vacuum and the residue dissolved in methylene chloride and purified on a column of florisil. Two fractions were obtained: 1) 4 mg, m.p. 145–147°C (ethyl acetate–hexane); mass spectrum (*m/e*); 248 (M). IR spectrum (in cm^{-1}): 1766, 1184 (γ-lactone) 1690, 1633 (conjugated ketone in a five-membered ring), 1408 ($—CH_2—$ near a keto group). 2) 6 mg m.p. 122–125°C (ethyl acetate–hexane); mass spectrum *m/e*: 248.

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