ON TERPENES. CCXIX.*
THE STRUCTURE OF GROSHEIMIN**

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On the basis of PMR spectra and optical properties of grosheimin and some of its derivatives the authors checked the correctness of the formula for grosheimin preliminarily proposed by Bretón and coworkers, and also proposed for it the relative and absolute configuration represented by formula *XXIII*. In connection with grosheimin some problems are discussed concerning the determination of the stereochemistry of guaian-6,12-olides and pseudoguaian-6,12-olides.

In connection with the study of sesquiterpenic lactones from *Compositae* we also investigated sesquiterpenic lactones from *Cynara scolymus* L. In addition to other substances we also isolated substance *I* of the composition $C_{15}H_{18}O_4$, m.p. 205°C and $[\alpha]_D^{20}$ +137·7°, from the leaves of this plant. Its IR spectrum contained absorption bands corresponding to a γ-lactone ring with an exomethylene double bond conjugated with the lactone carbonyl group (1755, 1145, and 1405 cm^{-1}), a keto-group in a five-membered ring (1735 cm^{-1}), a double bond (1645 cm^{-1}), and a hydroxyl group (3500 and 3600 cm^{-1}).

The infrared spectrum and the physical constants indicated that the isolated substance *I* is most probably identical with grosheimin which was originally isolated[1] from the leaves of *Grossheimia macrocephala* (Muss.-Puschk.) D. Sosn. et Takht. and later on by Spanish chemists[2] from *Amberboa Lippii* D. C. (synonymum *Centaurea Lippii* L.). The identity of substance *I* with grosheimin was proved by the isolation of grosheimin from *G. macrocephala* and comparison of their IR and PMR spectra, as well as other physical constants, which were identical. The mixture melting point of substance *I* with grosheimin from *G. macrocephala* was undepressed.

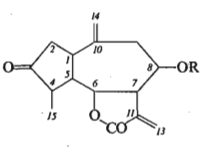
* Part CCXVIII: Parfums Cosmét. Savons, in press.

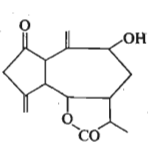
** After this paper had been sent to the editors, the paper by A. G. Gonzáles, B. García Marrero, and J. L. Bretón, published in Anales Quím. *66*, 799 (1970), became available to us, in which the stereostructure of several native guaianolides, grosheimin among them, is proposed.

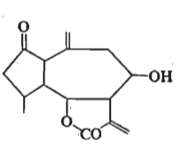
The structure of grosheimin was studied first by Rybalko and Sheichenko[3] who proposed for it the structural formula *II*; however, they soon corrected it to formula[4,5] *III*. Shortly afterwards Bretón and coworkers published a preliminary communication[6] in which they deduced the structure *I* for grosheimin on the basis of its chemical correlation with the sesquiterpenic lactone amberboin[2] (*IV*) and cynaropicrin[7,8].

In view of the fact that our present study of sesquiterpenic lactones of *C. scolymus*[9] indicates that the substance described originally as cynaropicrin[7,8] was probably a mixture of several sesquiterpenic lactones of which one might also have been grosheimin, we considered it necessary to check the structure of grosheimin on the basis of a detailed analysis of its PMR spectrum.

The PMR spectrum of grosheimin (*I*) (100 MHz; deuteriochloroform; Fig. 1) showed characteristic signals of one secondary methyl group as a doublet at 1·26 p.p.m. (J = 6·9 Hz; the signal of the corresponding methine proton was at ~2·30 p.p.m.), and signals of protons of two exomethylene groups (assigned on the basis of nuclear Overhauser effect), of which one formed two quartets at 6·36 p.p.m. and 6·31 p.p.m., typical of exomethylene protons of α,β-unsaturated γ-lactone ring[10] (low-field proton referred to as $H_{(13)}$, high-field proton as $H_{(13')}$, and the allylic proton as $H_{(7)}$; $^4J_{7,13}$ = 3·35 Hz; $^4J_{7,13'}$ = 3·00 Hz; $^2J_{13,13'}$ = 1·15 Hz) and the other two broadened singlets with incompletely resolved fine structure at 5·08 and 4·84 p.p.m., typical of protons of the unconjugated exomethylene double bond. The signal of proton $H_{(7)}$ was localised at 3·07 p.p.m. where it formed approximately a quartet of triplets (overlapping with multiplets of two other protons) in consequence of allylic interactions with the protons at $C_{(13)}$ and two vicinal couplings with two protons, of which one formed a triplet at 3·99 p.p.m. (J_1 = 9·5 Hz and J_2 = 8·9 Hz) and the other a complex multiplet at 3·91 p.p.m., which after addition of deuterioacetic acid changed to a quartet of doublets (J_1 = 5·8 Hz, J_2 = 8·7 Hz, and, J_3 = 9·9 Hz). Therefore, the first of the protons could be assigned to a proton of the —CH(—O—CO—$C_{(11)}$—)—$C_{(7)}$H– type, *i.e.* to a proton on the carbon carrying the ethereal oxygen of γ-lactone ring, and the second one to a proton of the —CH(—OH)—$C_{(7)}$H– type. Two of the splitting constants of the methine proton —CH—OH, J_1 = 5·8 and J_2 = 8·7 Hz, corresponded to the interactions with two protons forming a







I, R = H
VI, R = $COCH_3$
VII, R = $CONHCOCCl_3$
VIII, R = $COC_6H_3(NO_2)_2$

II *III*

quartet at 2·87 p.p.m. (J_1 = 5·8 Hz and $J_2 = {}^2J$ = 13·0 Hz) and a quartet of doublets at 2·30 p.p.m. (J_1 = 8·8 Hz, $J_2 = {}^2J$ = 13·0 Hz, J_3 = 0·9 Hz), and which could be assigned to the methylene group of the —C—CH_2—CH(OH)—$C_{(7)}$– type bound to a tetrasubstituted carbon atom (sp^3 or sp^2). Both methylene protons exhibited long-range couplings with the proton of the unconjugated exomethylene double bond, which formed a broadened singlet at 5·08 p.p.m. (Fig. 1). The second proton of this exomethylene group formed a broadened signal at 4·84 p.p.m. with an uncompletely resolved triplet structure caused by two dominating couplings, one geminal with $J_1 = {}^2J$ = 0·5 Hz and the other a long-range one with J_2 = 0·8 Hz, with another proton which formed a multiplet at ~3·14 p.p.m. (partially overlapping with the multiplet of $H_{(7)}$).

The irradiation of the position at 3·14 p.p.m. led both to the collapse of the doublet with the relative intensity 2 H centered at 2·52 p.p.m. (J = 7 Hz), and to the perturbation of multiplets in the region about 2·30 p.p.m. From this experiment it may be deduced that the corresponding proton interacts with three protons two of which gave the mentioned doublet as the X-part of the terminal subsystem of the XX'CB type with $^1/_2\,|J_{CX} + J_{CX'}| \cong 7$ Hz and $J_{BX} = J_{BX'} = 0$, if the proton with the shift at 3·14 p.p.m. is considered as proton C. In the region about 2·30 p.p.m. the multiplets of three protons are superimposed one of which is due to the proton of the

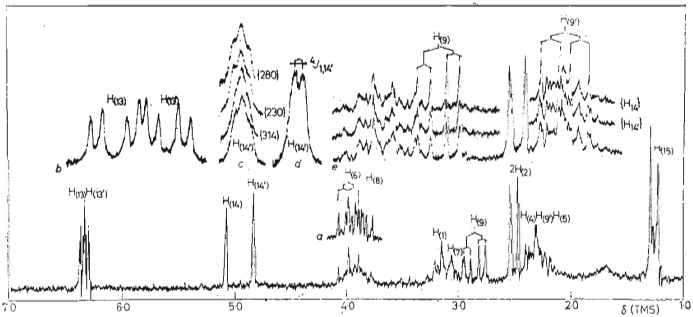


FIG. 1

PMR-Spectrum of Grosheimin (HA-100, Deuteriochloroform) and Some Decoupling Experiments (irradiated positions indicated as { })

a Multiplets of $H_{(6)}$ and $H_{(8)}$ after addition of trace of tetradeuterioacetic acid; *b* high-resolved multiplets of $C_{(13)}$-protons (50 Hz sweep width); *c* signal of $H_{(14')}$ on 50 Hz sweep width and decoupling experiments; *d* signal of $H_{(14')}$ after decoupling from $H_{(14)}$ (50 Hz sweep-width); *e* part of the basic spectrum (2·0—3·3 p.p.m.) on 250 sweep width and decoupling experiments.

methylene group of the —C$\underline{H}_2$—CH(OH)—$C_{(7)}$-type and the second to the methine proton of the —C$\underline{H}$—CH_3 type. Hence, the proton B is either the proton bound to the last of the as yet unidentified carbon atoms of the sp^3 type and corresponds to the third multiplet in the mentioned region, or it is identical with the proton of the —C$\underline{H}$—CH_3 type. As one of the splitting constants of the proton of the —C$\underline{H}$(—O—CO—$C_{(11)}$—$C_{(7)}$H-type corresponded to the coupling with the proton the multiplet of which also lies in the region at 2·30 p.p.m., and as the signal of the secondary methyl group formed a typical complex doublet of second order ABX_3 type, indicating that the methine proton is a part of a strongly coupled subsystem, the proton B may correspond either to the type CH_3—CH_A—CH_B—CH(—O—CO—$C_{(11)}$—)—$C_{(7)}$H— or CH_3—CH_B—CH_A—CH(—O—CO—$C_{(11)}$—)—$C_{(7)}$H—. This assignment of the protons A and B was confirmed by the PMR spectrum of grosheimin in benzene in which the methyl protons already formed a regular second order doublet without considerable "filling in". Simultaneously the proton B formed an already separated multiplet, enabling thus a decision on the basis of decoupling experiments in favour of the first of the two mentioned alternative structural types of proton B.

The observed topological continuity of vicinal and long-range couplings is consistent with the topology of the bonds of 8-hydroxy-3-oxo-guai-(10,14)-en(6,12)-olide, expressed by formula *I* and it supports the conclusion made by Bretón and coworkers[6]. The correctness of formula *I* for grosheimin was further corroborated by

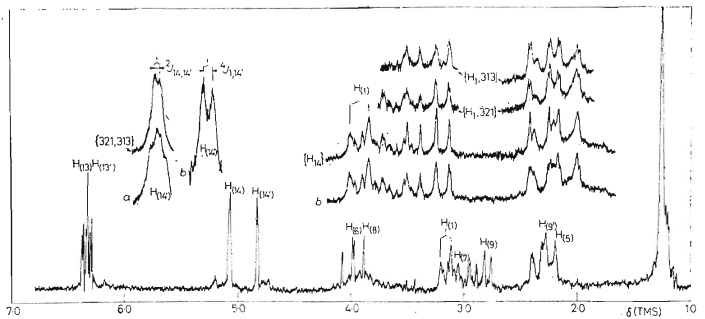


FIG. 2

PMR-Spectrum of Deuteriogrosheimin (HA-100, Deuteriochloroform) and Some Decoupling Experiments (irradiated positions indicated as { })

a Signal of $H_{(14')}$ on 50 Hz sweep-width and triple-resonance experiment $H_{(14')}$ — {$H_{(1)}$: : 321, 313}; *b* part of the basic spectrum (2·0—3·3 p.p.m.) on 250 sweep-width and some double resonance experiments.

direct deuteration. In the PMR spectrum of deuteriated grosheimin (*V*) in deuteriochloroform (Fig. 2) in agreement with the proposed formula the two-proton doublet at 2·52 p.p.m. which was assigned to the protons at $C_{(2)}$ was absent, and the signal of proton $H_{(1)}$ appeared as a broadened doublet at 3·17 p.p.m. with $J_{1,5} = 8{\cdot}0$ Hz and $J_{1,14'} = 0{\cdot}85$ Hz, as was confirmed by triple resonance experiment (Fig. 2). Simultaneously the multiplicity of the signals in the region about 2·30 p.p.m. and in the methyl region also changed after deuteriation. However, the relative intensities of these regions were larger than would correspond to structure *V* for deuteriated grosheimin (2 H at 2·30 p.p.m., 3 H at 1·26 p.p.m.) and they indicated the presence of impurities (intensive singlet at 1·26 p.p.m. in the spectrum of deuteriated grosheimin (Fig. 2) cannot be assigned unambiguously to the methyl protons of the —CD— —(CH_3)-type in view of its intensity and shape) in the measured sample of the deuterated grosheimin obtained. In spite of this circumstance the deuteriation of the second α-position ($C_{(4)}$) of the carbonyl group was confirmed by the presence of the quartet of proton $H_{(5)}$ indicated in the preceding discussion as proton B in the PMR spectrum of the solution of deuteriated grosheimin in benzene (Table I). The mentioned facts confirm clearly the structural formula *I* for the molecule of grosheimin.

We further investigated the stereochemistry of grosheimin for which the following conclusion can be drawn on the basis of the experimental material studied. From the comparison of the chemical shifts and geminal interaction of protons $H_{(13)}$ and $H_{(13')}$ in the PMR spectrum of grosheimin (*I*) and acetylgrosheimin (*VI*) (in deuteriochloroform) a paramagnetic shift of the signal of proton $H_{(13')}$ (cisoid) (Table I) $\Delta(\text{cisoid}) = \delta H_{(13')}(\text{OH}) - \delta H_{(13')}(\text{OCOCH}_3) = 0{\cdot}5$ p.p.m. and an increase of geminal coupling $\Delta^2 J = {}^2J(\text{OH}) - {}^2J(\text{OCOCH}_3) = 0{\cdot}65$ Hz followed, which indicated, according to present knowledge, the van der Waals effect of the oxygen atom of OR substituents at $C_{(8)}$, typical of 8-α-hydroxy-6,12-olides of various structural types[10]. The nature of this effect[10] was corroborated in the case of grosheimin by using acyl groups with a greater possibility of delocalisation of lone electron pairs of the ethereal oxygen of the O-acyl group, as for example in the adduct of grosheimin with trichloroacetylisocyanate (*VII*)* or in 3,5-dinitrobenzoate of grosheimin (*VIII*) where the residual geminal interaction ${}^2J = 0{\cdot}5$ Hz in acetyl grosheimin (*VI*) was further reduced to ${}^2J = 0$ (Table I). For the stereochemistry of guaian-6,12-olides and pseudoguaian-6,12-olides this effect[10] only indicates a quasi-parallelity of $C_{(8)}$—O and $C_{(11)}$= $=C_{(13)}$ bonds and therefore also the pseudodiequatorial conformation of $C_{(8)}$—O and $C_{(7)}$—$C_{(11)}$ bonds (pseudoequatorial position of the $C_{(7)}$—$C_{(11)}$ bond is determined *a priori* by the criterion of optimum conformations of the perhydroazulene sesquiterpenic skeleton requiring a diequatorial orientation of the five-membered ring in both cases of ring fusion, *cis* or *trans*[12]) as these may be achieved in suitable

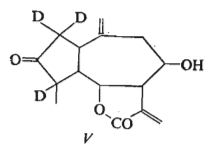
* The adduct *VII* was prepared by *in situ* reaction[11], on addition of a drop of trichloroacetylisocyanate to the original solution of grosheimin in deuteriochloroform, after acidification with a drop of deuteriated acetic acid and after a 1 : 2 dilution.

TABLE I

Parameters of the PMR Spectrum of Grosheimin (*I*), Deuteriated Grosheimin (*V*), Acetyl Grosheimin (*VI*), the Adduct of Grosheimin with Trichloroacetylisocyanate (*VII*), and 3,5-Dinitrobenzoate of Grosheimin (*VIII*)

Compound[a] (solvent)	$H_{(1)}$	$H_{(2)}$	$H_{(4)}$	$H_{(5)}$	$H_{(6)}$	$H_{(7)}$	$H_{(8)}$	$H_{(9)}$
I (A)	3·14[b]	2·52	2·30[b]	2·30[b]	3·99[c,d] $J_{6,5} = 9·5$ $J_{6,7} = 8·9$	3·07	3·91[c,d] $J_{8,9} = 5·8$ $J_{8,9'} = 8·7$ $J_{8,7} = 9·9$	2·87[d] $J_{9,8} = 5·6$ $J_{9,9'} = 13·0$
V (A)	3·17 $J_{1,5} = 8·0$	—	—	2·30	3·99[g] $J_{6,5} = 10·0$ $J_{6,7} = 8·8$	3·07[h] $J_{7,8} \approx 10$	3·94	2·86[g,d] $J_{9,8} = 5·8$ $J_{9,9'} = 13·0$ $J_{9,14} = 0·4$
I (B)	2·64[b]	2·15	2·07[b]	1·72[i] $J_{5,4} = 8·5$ $J_{5,1} = 8·5$ $J_{5,6} = 9·5$	3·44[c,e] $J_{6,5} = 9·8$ $J_{6,7} = 9·0$	2·84[j] $J_{7,8} \approx 10$ $J_{7,6} \approx 9$	3·60[c,e] $J_{8,9} = 5·7$ $J_{8,9'} = 8·7$ $J_{8,7} = 9·7$	2·73[g] $J_{9,8} = 5·8$ $J_{9,9'} = 12·8$
V (B)	2·65[b,l] $J_{1,5} = 8·5$	—	—	1·76[l] $J_{5,6} \approx 10$ $J_{5,1} \approx 8·5$	3·46[l] $J_{6,5} \approx 10$ $J_{6,7} \approx 9$	—	3·61	—
I (C)	3·20[b]	—	2·23[b]	—	4·04[d] $J_{6,5} = 9·5$ $J_{6,7} = 8·9$	3·08 $J_{7,6} = 8·8$ $J_{7,8} = 9·8$	3·69[c,d] $J_{8,7} = 9·7$ $J_{8,9} = 5·6$ $J_{8,9'} = 8·5$	2·71 $J_{9,8} = 5·8$ $J_{9,9'} = 12·8$
VI (A)	3·20[b]	2·52	2·30[b]	2·30[b]	4·06[l,p] $J_{6,5} = 9$ $J_{6,7} = 9$	3·28[b]	4·95 $J_{8,7} = 10$ $J_{8,9} = 5·5$ $J_{8,9'} = 9$	2·98[l] $J_{9,8} = 5·5$ $J_{9,9'} = 13$
VII[r] (A)	3·20	2·55	2·26	2·26	4·11[l] $J_{6,5} = 9·5$ $J_{6,7} = 8·5$	3·44[l,h] $J_{7,6} = 9$ $J_{7,8} = 10·5$	5·07[s]	3·10[l] $J_{9,8} = 6$ $J_{9,9'} = 13$
VIII[u] (A)	3·27	—	—	—	4·17[g] $J_{6,5} = 9·8$ $J_{6,7} = 9$	3·58[l,h] $J_{7,6} = 9$ $J_{7,8} = 10·5$	3·09[l] $J_{8,9} = 5·9$ $J_{8,9'} = 8·7$ $J_{8,7} = 10·6$	3·09[l] $J_{9,8} = 6$ $J_{9,9'} = 13$

Compound[a] (solvent)	$H_{(9')}$	$H_{(13)}$	$H_{(13')}$	$H_{(14)}$	$H_{(14')}$	$H_{(15)}$	other data
I	2·30[d]	6·36[e]	6·31[e]	5·08	4·84[f]	1·26[f]	$J_{14,9} \neq 0 \leqq 0{\cdot}5$
(A)	$J_{9',8} = 8{\cdot}8$	$J_{13,7} = 3{\cdot}35$	$J_{13',7} = 3{\cdot}0$		$J_{14',14} = 0{\cdot}5$	$J_{15,4} = 6{\cdot}9$	$J_{14',9} \leqq 0{\cdot}1$
	$J_{9',9} = 13{\cdot}0$	$J_{13,13'} = 1{\cdot}15$	$J_{13',13} = 1{\cdot}15$		$J_{14',1} = 0{\cdot}8$		$J_{14',9'} \leqq 0{\cdot}1$
	$J_{9',14} = 0{\cdot}9$						$^1/_2\lvert J_{1,2} + J_{1,2'}\rvert \approx 7$
V	2·31[g,d]	6·35[e]	6·31[e]	5·08	4·83[f,k]	1·26	$J_{14',9} \neq 0 \leqq 0{\cdot}1$
(A)	$J_{9',8} = 8{\cdot}5$	$^4J_{13,7} = 3{\cdot}26$	$^4J_{13',7} = 2{\cdot}96$		$J_{14',14} = 0{\cdot}4$		
	$J_{9',9} = 13$	$J_{13,13'} = 1{\cdot}10$	$J_{13',13} = 1{\cdot}10$		$J_{14',1} = 0{\cdot}85$		
	$J_{9',14} = 1$						
I	2·13[b]	[j,h]	[j,h]	4·78	4·50[f]	1·20[e]	$J_{9,14} \neq 0 < J_{9',14} \neq 0 \leqq 1$
(B)					$J_{14',14} = 0{\cdot}75$	$J_{15,1} = 6{\cdot}9$	OH: 5·02 $J_{OH,8} = 5{\cdot}5$
					$J_{14',1} = 0{\cdot}75$		$\lvert J_{7,13} + J_{7,13'}\rvert \approx 6{\cdot}5$
							$^1/_2\lvert J_{1,2} + J_{1,2'}\rvert \approx 7$
V	2·15[b]	[j,h]	[j,h]	4·80	4·50	1·17[m]	—
(B)	$J_{9',9} = 13$						
	$J_{9',8} = 9$						
I	—	6·23[e]	6·11[e]	4·98	4·96[f]	1·11[e]	OH: 5·30 $J_{OH,8} = 6$
(C)		$J_{13,7} = 3{\cdot}0$	$J_{13',7} = 3{\cdot}35$		$J_{14',14} = 1{\cdot}3$	$J_{15,4} = 6{\cdot}8$	$J_{14,9} \neq 0 \ll 0{\cdot}5$
		$J_{13,13'} = 1{\cdot}70$	$J_{13',13} = 1{\cdot}70$		$J_{14',1} = 0{\cdot}5$		$H_{(2)}$, $H_{(5)}$, $H_{(9')}$: 2·07–2·57
VI	2·23[l]	6·34[q]	5·82[q]	5·15	4·88	1·26[l]	$^1/_2\lvert J_{1,2} + J_{1,2'}\rvert \approx 7$
(A)	$J_{9',8} = 9$	$J_{13,7} = 3{\cdot}3$	$J_{13',7} = 3{\cdot}0$			$J_{15,4} = 6{\cdot}5$	$CH_3.CO$: 2·15
	$J_{9',9} = 13$	$J_{13,13'} = 0{\cdot}5$	$J_{13',13} = 0{\cdot}5$				
VII[r]	—	6·39[e,q]	5·97[e,q]	5·19[t]	4·93[t]	1·27[l]	$^1/_2\lvert J_{1,2} + J_{1,2'}\rvert \approx 7$
(A)		$J_{13,7} = 3{\cdot}25$	$J_{13',7} = 2{\cdot}90$			$J_{15,4} = 6{\cdot}5$	
		$J_{13,13'} = 0$	$J_{13',13} = 0$				
VIII[u]	—	6·29[e,q]	5·63[e,q]	5·22[t]	4·99[t]	1·30[e]	$J_{14,14'} \neq 0 < 0{\cdot}4$
(A)		$J_{13,7} = 3{\cdot}3$	$J_{13',7} = 2{\cdot}9$			$J_{15,4} = 7{\cdot}0$	$H_{(2)}$, $H_{(3)}$, $H_{(4)}$, $H_{(9)}$:
		$J_{13,13'} = 0$	$J_{13',13} = 0$				2·32–2·72



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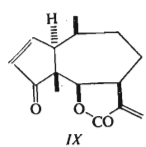

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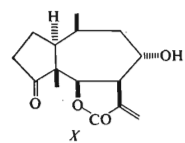
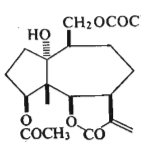


X



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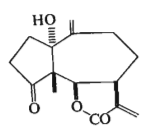
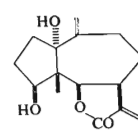
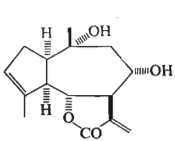


XII



XIII



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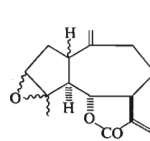
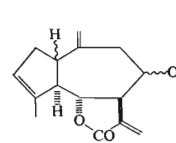


XV



XVI

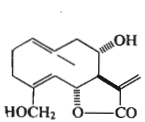
of Table I

[a]Measured on a Varian HA-100 instrument; solvents: A: deuteriochloroform; B: benzene with a small addition of hexadeuteriodimethyl sulfoxide; C: hexadeuteriodimethyl sulfoxide; first order analysis, chemical shifts δ(TMS) scale, splittings in Hz; [b]approximate position from decoupling experiments; [c]splittings measured after exchange with deuterioacetic acid; [d] splittings measured on 100 Hz sweep width chart; [e]counter measurement of splittings on 50 and 100 Hz sweep width chart (possible error $\pm 0{\cdot}1$ Hz; [f] splittings obtained by comparison of multiplets $H_{(14')}$ and $H_{(14')} - \{H_{(14)}\}$ on 100 Hz sweep width chart (irradiated position indicated as { }); [g] splittings from 250 Hz sweep width chart; [h]splittings from multiplet of $H_{(7)}$; [i]separate multiplet with $\Sigma J =$ $= 26{\cdot}5$ Hz analysed as triplet of doublets; $^{j}|^{4}J_{7,13} + {}^{4}J_{7,13'}| \approx 6{,}5$ Hz (in this solution the signals of protons on $C_{(13)}$ overlapped by strong signal of benzene-protons); [k] $^{2}J_{14,14'}$ measured as doublet splitting in the multiplet $H_{(14')} - \{H_1^{(1)}, H_1^{(2)}\}$; [l]splittings from the 500 Hz sweep width chart; [m] position of the methyl signal uncertain due to impurities forming signals at 1·17, 1·23, 1·27 and 1·29 p.p.m; despite this circumstance the position of deuterium on $C_{(4)}$ was clearly demonstrated by the quartet of $H_{(5)}$; [n] splittings from the multiplet $H_{(7)} - \{H_{(13)}; H_{(13')}\}$; [p] second order triplet $\Sigma J \approx 18$ Hz; [q] geminal coupling confirmed by $H_{(13)} - \{H_{(13')}\}$ experiment; [r] measured after 1 : 2 dilution of the original solution (acidified with deuterioacetic acid) and addition of trichloroacetyl isocyanate; [s] splittings not estimable because of coincidence of the outer lines of the $H_{(8)}$-multiplet with $H_{(14)}$ and $H_{(14')}$ signals; [t] splittings not resolved; [u] adduct with one molecule of toluene, phenyl protons at 7·18 p.p.m., methyl protons at 2·34 p.p.m.

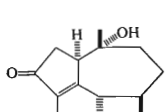
conformations of the seven-membered ring in both relative configurations 7α-H, 8α-OH, and 7α-H, 8β-OH. The pseudodiequatorial conformation of $C_{(8)}$—O and $C_{(7)}$—$C_{(11)}$ bonds is also in agreement with the observed splittings $J_{7,8} = 9{\cdot}5 - 10{\cdot}5$ Hz formed in the PMR spectrum of grosheimin (*I*) and its derivatives *VI* to *VIII* (Table I), which, in principle, may correspond to *syn*- or *anti*periplanar configuration of protons $H_{(7)}$ and $H_{(8)}$.

From the comparison of molecular rotations of grosheimin (*I*) ($[\Phi]_D + 301°$) and its 3,5-dinitrobenzoate *VIII* ($[\Phi]_D + 514{\cdot}5°$)* it followed that the increment is positive ($+213°$), which is according to the benzoate rule[13–16] an indication of the *S*-configuration of the center $C_{(8)}$ in grosheimin. From the pseudodiequatorial conformation of the $C_{(8)}$—O and $C_{(7)}$—$C_{(11)}$ bond and the *S*-configuration of the $C_{(8)}$ centre the absolute configuration 7α-H, 8β-H followed.

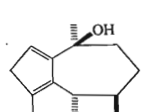
The observed values of allylic couplings of protons at $C_{(13)}$ $^4J_{7,13} = 3{\cdot}3 \pm 0{\cdot}1$ and $^4J_{7,13'} = 3{\cdot}0 \pm 0{\cdot}1$ Hz in grosheimin (*I*) and its derivatives *VI* to *VIII* (Table I) indicate, according to the empirical rule $|^4J|$ (*trans*-lactone[17]) $\geqq 3$ Hz, that the fusion of the lactone ring in the mentioned substances is probably *trans*.



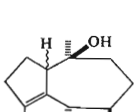
XVII



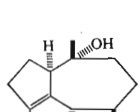
XVIII



XIX



XX



XXI

From the present data it follows that the mentioned rule[17] applies in the majority of sesquiterpenic lactones of various structural types. Considerable deviations appear mainly in pseudoguaian-6,12-olides which have a *cis*-fused lactone ring and where allylic coupling constants of the order 3—4 Hz[18–23] appear. These deviations are caused obviously by conformational factors and the transmission of non-bonding effects of substituents *via* the angular methyl group bound to $C_{(5)}$ (for example: transannular interactions $C_{(5)}$- methyl group and $H_{(8)}$ and $H_{(9)}$ in the $C_{(10)}$-chair-like or boat-like conformation) and they are due to the fact that in these cases the

* The molecular rotation is calculated for the adduct of 3,5-dinitrobenzoate of grosheimin (*VIII*) with one molecule of toluene, which was formed on crystallisation of the mentioned compound *VIII* from toluene. The adduct was proved by elemental analysis and the PMR spectrum.

conformation of the lactone ring need not always be the determining factor of the conformation of the seven-membered carbon ring. One of the possible explanations is the change of conformation of the γ-lactone ring from α-envelope ($C_{(7)}$, $C_{(11)}$, $C_{(12)}$ and $O_{(6)}$ in the plane, $C_{(6)}$ below the ring plane) to β-envelope ($C_{(6)}$ above the plane of the ring). For example in ambrosin[24] (*IX*) the quasiplanar cyclopentene ring is best realised in the $C_{(10)}$-chair-like conformation, and together with the non-bonding repulsions of the $C_{(5)}$-methyl group it prefers the α-envelope with a small dihedral angle (~ 60°C) and hence also small allylic coupling constants (2·0 Hz), in accordance with the rule[17]. In sesquiterpenic lactones with a saturated five-membered homocyclic ring the pseudorotation around the $C_{(1)}$—$C_{(5)}$ bond, together with the repulsions of the angular methyl group, enable a variation to the conformations lying between the planar form and the β-envelope with dihedral angles of 60—90°, which should be typical of *trans*-lactones[17]. This role of conformational factors is clearly demonstrated in Table II where the data of the PMR spectra of ambrosin[24] (*IX*), deacetylconfertiflorin[25] (*X*), tetraneurin-F[21] (*XI*), 10,14-dehydrocoronopillin[21] (*XII*), and $\Delta^{10(14)}$-deacetylanhydrotetraneurin-E[21] (*XIII*) are compared.

In the case of native guaian-6,12-olides with a defined stereochemistry and an exomethylene double bond in the lactone ring only *trans*-lactones were described so far, as for example cumambrin-B[10,26] (*XIV*), estafiatin[27] (*XV*), and ligustrin[28] (*XVI*),* for which the mentioned rule holds. In view of these circumstance it is presently impossible to answer the question to what extent the mentioned variability of allylic coupling constants of protons at $C_{(13)}$ is also characteristic for guaian-6,12-olides. Therefore, in the case of grosheimin the magnitude of allylic couplings of protons at $C_{(13)}$ alone does not unambiguously exclude a *cis*-anellation of the lactone ring. Unfortunately, the observed splittings $J_{6,7} = 8{\cdot}5-9$ Hz in the PMR spectra of grosheimin (*I*) and its derivatives *VI*—*VIII* (Table I) also do not permit a safer differentiation between the *cis*- and *trans*-fusion of the lactone ring[29,30].

Our measurements of the PMR spectra of deacetylconfertiflorin (*X*) and cumambrin-B (*XIV*)** also indicated the lability of the electronic structure of the lactone ring in 5β-methyl, 6α-H, 7α-H pseudoguaianolides. In contrast to cumambrin-B the increase in polarity of the solvent in the case of desacetylconfertiflorin together with an increase of geminal interaction and a decrease of the internal chemical shift of $C_{(13)}$ protons also caused an appreciable increase of allylic coupling constants. This indicates rather a change in conformation of the lactone ring (in the above mentioned sense), induced by the reaction electric field, than the pure solvent-effect as observed *e.g.* in the case of the geminal coupling. In grosheimin (*I*) the same effect was observed as earlier in salonitenolide[31,32] (*XVII*). In the PMR spectrum of grosheimin (*I*) in hexadeuteriodimethyl sulfoxide the low-field quartet of the $C_{(13)}$ proton displayed a larger allylic coupling constant than the quartet of the $H_{(13')}$ at a higher field. Equal magnitudes of allylic coupling constants as in deuteriochloroform (Table II) indicated that in the case of grosheimin the assignment of the signals of $C_{(13)}$ protons in hexadeuteriodimethyl sulfoxide solution, is more correctly performed on the basis of coupling constants than on the basis of chemical shift[10,17,33].

* To the hydroxyl group at $C_{(8)}$ in ligustrin β-orientation may be assigned on the basis of the absence of geminal interaction of protons at $C_{(13)}$, according to ref.[10].

** We thank Dr H. Yoshioka for the kind gift of the samples.

The inversion of the chemical shifts ($H_{(13')}$ (cisoid) at a lower field) is also in agreement with the observed effects in cumambrin-B (*XVI*) and deacetylconfertiflorin (*X*) (Table II). In the latter cases the change of solvents from deuteriochloroform to sulfoxide caused a diamagnetic shift of the low-field proton signal (which was assigned in agreement with the allylic coupling rule to the transoid proton $H_{(13)}$ by approximately 0·20 p.p.m., and of the up-field proton signal (cisoid proton) by approximately 0·07 p.p.m. An opposite assignment of protons at $C_{(13)}$ in the case of grosheimin (*I*) leads to practically the same values of the diamagnetic shift 0·25 p.p.m. for H (transoid) and 0·08 p.p.m. for H (cisoid). Preliminary expreriments with grosheimin showed that an inversion of chemical shifts takes place after addition of only one drop of hexadeuteriodimethyl sulfoxide to a solution in deuteriochloroform, and indicated that association phenomena are obviously involved which might serve for further characterisation of the electronic structure of the lactone chromophor. We were unable to prove the assignment of $C_{(13)}$ protons in hexadeuteriodimethyl sulfoxide by means of nuclear Overhauser effect between the proton of the hydroxyl group and $H_{(13')}$ (cisoid) proton. However, the observability of nuclear Overhauser effect may be connected in this case with the question of the conformation of the hydroxyl

TABLE II

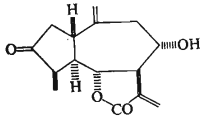
Structural and Solvent Dependence of PMR Parameters (first order values) of $C_{(13)}$-Exomethylene Protons in some Pseudoguaian-6,12-olides and Guaian-6,12-olides

Chemical shifts refer to tetramethylsilane as internal standard.

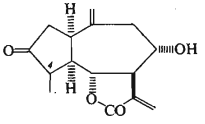
Compound	Solvent[a]	$H_{(13)}$ (transoid)	$^4J_{7,13}$	$H_{(13')}$ (cisoid)	$^4J_{7,13'}$	$^2J_{13,13'}$
Ambrosin[24] (*IX*)	A[ab]	6·29	2·0	5·50	2·0	[c]
Tetraneurin-F[21] (*XI*)	A[ab]	6·20	3·5	5·50	3·0	[c]
10,14-Dehydrocoronopilin[21] (*XII*)	A[ab]	6·23	2·3	5·63	1·8	[c]
$\Delta^{10(14)}$Deacetylanhydrotetraneurin-E[21] (*XIII*)	A[ab]	6·24	3·5	5·53	3·0	[c]
Deacetylconfertiflorin[25] (*X*)	A[d]	6·32	2·9	5·95	2·6	0·7
Deacetylconfertiflorin[25] (*X*)	B[d]	6·01	3·3	5·88	3·1	1·2
Cumambrin-B[10,26] (*XIV*)	A[d]	6·19	3·6	6·03	3·2	0·7
Cumambrin-B[10,26] (*XIV*)	B[d,e]	5·99	3·5	5·97	3·2	1·45
Grosheimin (*I*)	A[f]	6·36	3·35	6·31	3·0	1·15
Grosheimin (*I*)	B[f]	6·11	3·35	6·23	3·0	1·70

[a] A deuteriochloroform; B hexadeuteriodimethyl sulphoxide; [b] literature data; [c] zero or very small value, not confirmed by double resonance; [d] own measurements (HA-100) with frequency-counter on 50 or 100 Hz sweep-width (possible error ±0·1 to ±0·2 Hz); [e] inaccurate splitting-value due to strong overlapping of both quartets; [f] values from Table I.

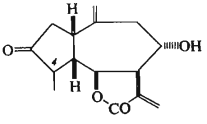
group. In hexadeuteriodimethyl sulfoxide the increase of temperature to 80°C also did not lead to any significant changes of the parameters of protons at $C_{(13)}$ or other protons; the only changes observed were an up-field shift of the proton signal of the hydroxy group (without a change of the coupling constant) and a distinct improvement of the spectrum resolution.



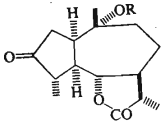
XXII



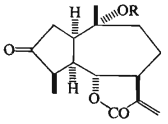
XXIII, 4αCH$_3$
XXIV, 4βCH$_3$



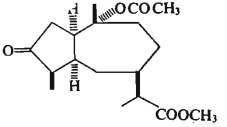
XXV, 4αCH$_3$
XXVI, 4βCH$_3$



XXVII, R = H
XXVIII, R = COCH$_3$



XXIX, R = H
XXX, R = COCH$_3$



XXXI

On the other hand the possibility of a *cis*-fusion of the lactone ring in the molecule of grosheimin (*I*) was indicated directly by its CD-curve (measured in methanol), which displayed ellipticity $[\Theta]_{255\cdot5} + 2376$ (without maximum) which indicates according to the postulated rules[34] for sesquiterpenic $C_{(6)}$-lactones (CE positive for *cis* lactone and CE negative for *trans* lactone fusion) a *cis* lactone; however, in view of the fact that the CD curve of grosheimin did not have a maximum at 255 nm, the conclusion drawn by this method cannot be considered as unambiguous. The question of the validity of the rules derived from the CD-curves for guaian-6,12-olides and pseudoguaian-6,12-olides is problematic as is the question of the validity of the rules on allylic couplings of $C_{(13)}$ protons. It is interesting that numerous exceptions from the CD-rules also occur in pseudoguaian-6,12-olides[34].

The problem of *cis-trans* isomerism of the lactone ring in guaian-6,12-olides depends to an appreciable extent on the relative stereochemistry of the $C_{(5)}$—$C_{(6)}$ fragment. This dependence is caused by the fact that in these substances the realisation of the pseudodiequatorial conformation of the $C_{(6)}$—O and $C_{(7)}$—$C_{(11)}$ bonds, which should determine a *priori* the most advantageous orientation of the γ-lactone ring, does not lead to optimum conformations with simultaneous pseudodiequatorial orientation of the five-membered homocyclic ring[12] for all possible combinations of relative configurations on the $C_{(5)}$, $C_{(6)}$, and $C_{(7)}$ centra. From the study of Dreiding models it can be deduced that the optimum conformation of pseudoguaian-6,12-

olides and guaian-6,12-olides with a pseudodiequatorial position of the *cis*-lactone ring may be realised in relative configurations 5-βH, 6-αH, and 7α-H, and with a pseudodiequatorial position of the *trans*-lactone ring in relative configurations 5α-H, 6β-H, and 7α-H. Interestingly, this conformational selectivity is in agreement with the present experimental material which shows that for native 7α-H pseudoguaian-6,12-olides 5β-configuration of the methyl group and *cis*-fusion of the lactone ring are typical, while for native 7α-H guaian-6,12-olides 5α-H configuration and *trans*-fusion of the lactone ring are typical. In the case of guaian-6,12-olides this conformational selectivity is also supported by the results of hydrogenation of isophoto-α-santonin lactone[35,36] (*XVIII*), artabsin[36] (*XIX*), dihydroartabsin[36] (*XX*) and 3-deoxo-isophoto-α-santonin lactone[36] (*XXI*); in all these cases substances with 5α-H configuration were obtained predominantly.

This consistence indicates the probability that in addition to structural selectivity a stereoselectivity of the mechanisms of biogenesis of guaian-6,12-olides and pseudoguaian-6,12-olides also exist simultaneously. This biogenetical stereoselectivity due to conformational factors is further supported indirectly by the fact that native $C_{(8)}$-lactones, where the realisation of the pseudodiequatorial conformation of $C_{(7)}$—$C_{(11)}$ and $C_{(8)}$—O bonds does not depend on the configuration of the $C_{(5)}$-center, occur in *cis* and in *trans*-fusion in one plant species[37] simultaneously. Extrapolation of the importance of conformational factors to the biogenesis of 8-hydroxyguaian-6,12-olides and 8-hydroxypseudoguaian-6,12-olides and to the problem of the stereochemistry of the fragment $C_{(7)}$—$C_{(8)}$ leads, under the supposition of an intermediary existence of a $C_{(8)}$-cation with an already defined stereochemistry of the $C_{(5)}$, $C_{(6)}$, and $C_{(7)}$ centra, to a preference of the staggered conformation of the $C_{(7)}$—$C_{(8)}$ fragment because a *syn*-periplanar arrangement of bonds in 8-hydroxy derivatives is an *a priori* restrictive factor. From this point of view – which, however, should also apply for all types of sesquiterpenic 8-hydroxy-6,12-olides – an equatorial position of the hydroxy group should be typical of 8α-hydroxy derivatives and an axial position of the hydroxyl of 8β-hydroxy derivatives. This stereochemical implication is in complete agreement with the finding of a selective occurrence of the mentioned van der Waals effect between the oxygen atom of the hydroxy group and $H_{(13)}$ proton (cisoid) in all types of 8α-hydroxy-7α-H 6,12-olides[10].

The application of these conclusions to grosheimin (*I*) with an *S*-configuration of the $C_{(8)}$ center leads to the supposition of two configurational sequences, *i.e.* a "guaian-6,12-olidic" one with 8β-H, 7α-H, 6β-H, and 5α-H, or a "pseudoguaian-6,12-olidic" one with 8β-H, 7α-H, 6α-H, and 5β-H. Both these sequences are consistent with both the preceding conclusions and also with the determined magnitude of the vicinal coupling $J_{5,6} = 9{\cdot}5 - 10$ Hz in the PMR spectra of grosheimin (*I*) and its derivatives *VI*–*VIII* (Table 1); in the case of the $C_{(5)}$–$C_{(6)}$ fragment this coupling indicates an *anti*-periplanar configuration of $H_{(6)}$ and $H_{(5)}$ protons. From the point of view of the considered structural and stereochemical selectivity of the biogenesis

of guaian-6,12-olides the first alternative may be preferred. The probability of this alternative further follows from the following data: the ORD curves of grosheimin (*I*) and acetylgrosheimin (*VI*) displayed a relatively weak positive Cotton effect at 295 nm (grosheimin: $a + 92{\cdot}4$; acetylgrosgheimin: $a + 74{\cdot}3$) due to the carbonyl chromophore in a five-membered homocyclic ring. As it follows from the earlier ORD studies[38-42] of substances with an analogous cyclopentanone ring which is in *cis*- or *trans*-fusion with the six- or seven-membered ring, in the case of *trans* fusion the sign and the amplitude of CE is determined by the conformation of the five-membered ring and hence also by absolute configuration of carbon atoms joining both cycles. When indicating – in analogy to grosheimin (*I*) – the carbon atoms at the ring junctions of the cyclopentane ring as $C_{(1)}$ and $C_{(5)}$, then the half-chair conformations of the cyclopentanone ring with carbons $C_{(1)}$ and $C_{(5)}$ either in negative or positive octants correspond to *trans*-fused 3-oxo derivatives and in agreement with the octant rule, to a negative or positive Cotton effect[38,41]. In these *trans*-fused 3-oxo derivatives amplitudes $a = 150-200$[38,40-42] were observed. From this point of view and under the supposition of a *trans* fusion of the rings in the case of grosheimin (*I*) a "positive" half-chair conformation of the cyclopentanone ring with a negative contribution of the $C_{(4)}$ methyl group results on the basis of the sign and the amplitude of the Cotton effect. Hence, the absolute configuration of centra at $C_{(1)}$, $C_{(4)}$ and $C_{(5)}$, and in view of the preceding conclusions also the absolute stereo structure expressed by formula *XXII* follow. In the case of *cis*-fused 3-oxo compounds the "positive" or "negative" half-chair conformation of the cyclopentanone may be realized in both possible absolute configurations of the centra[41] at $C_{(1)}$ and $C_{(5)}$. From this point of view and under the supposition of a *cis*-fusion of the five-membered and seven-membered rings, all corresponding combinations of absolute configurations of the centra $C_{(1)}$, $C_{(4)}$ and $C_{(5)}$ are *a priori* possible for the molecule of grosheimin, and hence, taking into account the preceding conclusions, also all alternative stereo-structures expressed by formulae *XXIII–XXVI*. Some aspects of the differentiation between possibilities *XXII–XXVI* follows from the comparison with dihydro derivatives of isophoto-α-santonin lactone *XXVII–XXX*. The ORD curves of these derivatives (*XXVII–XXX*) show the dependence of the sign of Cotton effect on the configuration of the $C_{(4)}$ methyl group: $a + 87{\cdot}5$ for *XXVII*, $a - 70$ for *XXIX*, $a + 25$ (ref.[43]) for *XXVIII*, and $a - 112$ for compound *XXX*. In the case of acetyl derivatives the difference of conformations of the cyclopentane ring was deduced on the basis of amplitude differences, and the interpretation of ORD was based in the case of substance *XXVIII* on the supposition of a quasi-planar conformation and of the sign being determined by the configuration of the methyl group, and in the case of substance *XXX* on the supposition of a negative half-chair conformation[43]. However, the ORD curves of hydroxy derivatives *XXVII* and *XXIX* indicated that the difference in amplitudes of ORD curves of acetyl derivatives is rather a consequence of a negative contribution of the acetyl group to the molar rotation than

a direct consequence of different conformations. This is also supported by the differences observed in the molar rotations of hydroxy derivatives *XXVII* and *XXIX* and the corresponding acetates *XXVIII* and *XXX* at 589 nm in chloroform: $[\Phi]_D$ (*XXVIII*) − $[\Phi]_D$ (*XXVII*) = − 174 and $[\Phi]_D$ (*XXX*) − $[\Phi]_D$ (*XXIX*) = −49 ($[\alpha]_D$(*XXVIII*) − 26° (refs[35,44]; $[\alpha]_D$(*XXVII*) +39° (refs[35,36]); $[\alpha]_D$(*XXX*) − 61·7° (ref.[44]); $[\alpha]_D$ (*XXIX*) − 46° (ref.[35]); the comparison of the ORD curves of grosheimin (*I*) and acetylgrosheimin (*VI*) also support this conclusion. Interpretation of the ORD curves of dihydro derivatives of isophoto-α-santonin lactone may be carried out either under the supposition that the sign of the Cotton effect is determined by the configuration of $C_{(4)}$ center and that the contribution of the anellation only changes the amplitude, or under the supposition that the Cotton effect sign is determined by the anellation and that the contribution of the $C_{(4)}$ center changes the amplitude. In view of the fact that in the second case the conformation of the cyclopentanone is changed in dependence on the configuration of the methyl group at $C_{(4)}$, both alternatives lead to the determination of the sign of the Cotton effect according to the absolute configuration of the methyl group at $C_{(4)}$ and *vice versa*. The ORD curves of grosheimin (*I*) and acetylgrosheimin (*VI*) (Fig. 3) are very similar with regard to their amplitude and fine structure to the ORD curve of hydroxy derivative *XXVII* (Fig. 3). The CD curve of grosheimin (*I*) ($[\Theta]_{296}$ +7458) was also very similar to the CD curve of substance *XXVII* ($[\Theta]_{296}$ + 7392) in the region about 300 nm. In view of this similarity the supposition of a *cis*-fusion of the five-membered and seven-membered carbon ring in grosheimin leads to the preference of the absolute stereostructure *XXIII* which has an identical configuration of the center at $C_{(1)}$, $C_{(4)}$ and $C_{(5)}$ as substance *XXVII*, and also of the alternative *XXV* which is pseudo-enantiomeric with substance *XXIX*.

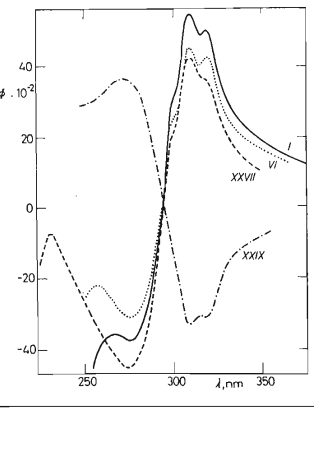


FIG. 3

Comparison of Cotton-Curves of the Cyclopentanone Chromophore in Grosheimin (*I*), Acetylgrosheimin (*VI*), 4α-Methyl-5αH-dihydroisophoto-α-santonin Lactone (*XXVII*) and 4β-Methyl-5αH-dihydroisophoto-α-santonin Lactone (*XXIX*)

All curves measured in methanolic solution.

Further differentiation between the remaining possible stereostructures *XXII*, *XXIII*, and *XXV* may be carried out from the point of view of the stability of the methyl group at $C_{(4)}$. As was found by dihydro derivatives of isophoto-α-santonin lactone, the position of the methyl group is very unstable in the case of β-configuration (*XXIX* and *XXX*), so that an easy inversion of the configuration may be observed even under the conditions of chromatography on alumina[35,36,43,44]. Easy epimerisation of the $C_{(4)}$ center has also been observed in the case of substance[42] *XXXI*. These examples indicated that the stability of the methyl group at $C_{(4)}$ is in the case of 3-oxo derivatives of perhydroguaiazulene determined *a priori* by vicinal steric effects (repulsion of the bonding orbitals of the $C_{(4)}$—CH_3 and $C_{(5)}$—$C_{(6)}$ bonds). Analogously an unstable position of the methyl group might be expected in the case of substances *XXII* and *XXV*. However, deuteriation of grosheimin, carried out in alkaline medium evidently took place without the inversion of configuration of the $C_{(4)}$ center, as indicated by the correspondence of the PMR spectra of grosheimin (*I*) and deuteriogrosheimin (*V*). In the PMR spectra of dihydro derivatives of isophoto-α-santonin lactone *XXVII*—*XXX* the change of configuration at the $C_{(4)}$ center is directly reflected by chemical shifts of the $H_{(6)}$ proton[36,44]. Hence, the position of the secondary methyl group in the molecule of grosheimin is thermodynamically stable. From this point of view the alternatives *XXII* and *XXV* may be considered as unlike.

Taking into consideration the sum of all aspects, which were obtained on the basis of the present experimental material, the absolute stereostructure expressed by formula *XXIII* can be proposed for grosheimin.

EXPERIMENTAL

Melting points were determined on a Kofler block and were not corrected. For column chromatography silica gel according to Pitra and Štěrba[45] was used (particle size 30—60 μ, deactivated by addition of 11% of water); for thin-layer chromatography silica gel with gypsum according to Stahl was employed. The IR spectra were measured in chloroform on a Unicam S. P. 200 spectrophotometer. The PMR spectra were measured with a Varian HA-100 apparatus. Mass spectra were measured with a spectrograph AEI MS 902. Optical rotation and optical rotatory dispersion were determined on a Jasco UV-5 spectropolarimeter. Circular dichroism was measured on a Roussel-Jouan Dichrographe CD-185.

Isolation of Grosheimin

A) *From* Cynara scolymus L.: Freshly ground leaves (20 kg) of Italian origin were extracted with acetone at room temperature. After evaporation of the solvent at 25°C under reduced pressure a residue was obtained which was partitioned between light petroleum (1 l) and water (1 l). The aqueous phase was extracted with two 1 litre portions of light petroleum and four times with chloroform (0·5 l each shaking). The residue of the chloroform extract (34 g) was chromatographed on 750 g of silica gel with light petroleum and ethyl acetate. A 1 : 1 mixture of the mentioned solvents eluted crude grosheimin which was purified by repeated chromatography on silica gel, using a chloroform–methanol mixture (49 : 1) for elution. Grosheimin (*I*) of m.p. 197°C (ethyl acetate) was obtained, $[\alpha]_D^{20}$ +123° (*c* 1·0; chloroform). For $C_{15}H_{18}O_4$ (262·3) calculated: 68·68% C, 6·92% H, 0·38% H act.; found: 68·82% C, 7·08% H, 0·45% H act. ORD: $[\Phi]_{400}$ +964, $[\Phi]_{320}$ +4985°, $[\Phi]_{317}$ +4876°, $[\Phi]_{309}$ +5495°, $[\Phi]_{295}$ ±0°, $[\Phi]_{275}$ — — 3740°, $[\Phi]_{265}$ −3540°. CD: $[\Theta]_{330}$ ±0°, $[\Theta]_{296}$ +7458°, $[\Theta]_{244}$ ±0°, $[\Theta]_{221}$ −20360°.

B) *From* G. macrocephala. Dry ground leaves (3·0 kg) of *G. macrocephala* (MUSS. - PUSCHK.) D.SOSN. et TAKHT. species cultivated in Pharmacognostical Garden, Poznań (Poland), and collected before flowering were worked up in a similar manner as before[37] and a residue was obtained (23 g) which contained according to thin-layer chromatography three substances of R_F values 0·43, 0·60 and 0·90 (in chloroform–acetone 3 : 1). The extract (20 g) was chromatographed on 500 g of silica gel with chloroform and then chloroform and acetone (3 : 1). From the medium fractions grosheimin has been isolated, m.p. 205°C (methanol), $[\alpha]_D^{20}$ +137·7° (*c* 0·225; methanol), R_F 0·60 (chloroform–acetone 3 : 1). For $C_{15}H_{18}O_4$ (262·3) calculated: 68·68% C, 6·92% H, 0·38% H act.; found: 68·71% C, 6·90% H, 0·39% H act.

Deuteriation: Grosheimin (*I*; 19·1 mg) was mixed with 2 ml of deuterium oxide and 2 ml of deuterioethanol, 10 mg of potassium hydroxide were added, and further procedure was as described elsewhere[46]. After filtration through silica gel a product was obtained (*V*), yield 9 mg, *m/e* 265.

Acetyl Grosheimin *VI*

Grosheimin (*I*; 500 mg) was acetylated in the conventional manner to give *VI* of m.p. 149—151°C and R_F 0·70 (chloroform–acetone 9 : 1). ORD (methanol): $[\Phi]_{400}$ +1091°, $[\Phi]_{320}$ +4985°, $[\Phi]_{317}$ +4045°, $[\Phi]_{310}$ +4550°, $[\Phi]_{294}$ ± 0°, $[\Phi]_{275}$ −3080°, $[\Phi]_{257}$ −2180°.

3,5-Dinitrobenzoate of Grosheimin (*VIII*)

A mixture of grosheimin (*I*; 216 mg) in 1 ml of pyridine and 200 mg of 3,5-dinitrobenzoyl chloride in 1 ml of benzene was allowed to react at room remperature for 5·5 hours. After the conventional working up ester *VIII* was obtained of m.p. 85°C (toluene) and $[\alpha]_D^{25}$ +93·8° (*c*. 0·5; methanol). For $C_{22}H_{20}N_2O_9.C_7H_8$ (548·5) calculated: 63·50% C, 5·14% H, 5·10% N; found: 63·36% C, 5·09% H, 5·04% N.

4α-Methyl-5αH-dihydroisophotosantonin Lactone (*XXVII*) (ref.[36])

ORD: $[\Phi]_{400}$ +365°, $[\Phi]_{320}$ +3650°, $[\Phi]_{317}$ +3577°, $[\Phi]_{310}$ +4230°, $[\Phi]_{296}$ ±0°, $[\Phi]_{274}$ − −4520°, $[\Phi]_{230}$ −840°. CD: $[\Theta]_{330}$ ± 0°, $[\Theta]_{296}$ +7392°, $[\Theta]_{247}$ +330°, $[\Theta]_{216}$ +4422°.

4β-Methyl-5αH-dihydroisophotosantonin Lactone (*XXIX*) (ref.[36])

ORD: $[\Phi]_{400}$ −420°, $[\Phi]_{318}$ −3150°, $[\Phi]_{315}$ −3080°, $[\Phi]_{308}$ −3360°, $[\Theta]_{295}$ ± 0°, $[\Phi]_{275}$ +3640°.

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