TWO CRYSTALLINE FORMS OF SESQUITERPENE LACTONE HELENALIN – X-RAY AND NMR STUDY*,**

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Helenalin, a naturally occurring sesquiterpene lactone of the pseudoguaianolide type, has been reported to exist in two polymorphic forms, both crystallizing in the monoclinic space group $P2_1$. In this paper we report the X-ray structure of the third room temperature polymorph, crystallizing in the orthorhombic space group $P2_12_12_1$. The isolated molecules in all three polymorphs exhibit nearly the same molecular conformation, so the three crystalline modifications differ mainly in the mode the molecules pack in the solid state. Two main hydrogen bond acceptors, the oxygen atom of the cyclopentenone ring and the oxygen atom of the lactone carbonyl group, are competing in the hydrogen bond formation with the secondary hydroxyl group. Having an access to two polymorphs which differ in the hydrogen bond acceptors, we have performed the solid state ^{13}C NMR measurements using a cross-polarization magic angle spinning technique. By comparison of the spectra taken in non-polar solvent with the spectra taken in the solid state we were able to demonstrate the shielding effects resulting from the intermolecular hydrogen bonding in crystals. The fast conformational equilibrium between two conformers *TC*(10) and *TC*(7) was suggested from NMR study of helenalin in solution.

Helenalin (*I*), the major constituent of several *Helenium* species[1] is a sesquiterpene lactone of pseudoguaianolide type (5,7-ring skeleton, methyl group at C(5) ring junction). It is a representative of the helenanolide subgroup of pseudoguaianolides in which the lactone ring is attached at C(7) and C(8) and the C(10) methyl group is α.

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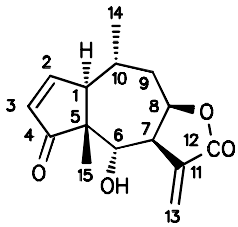
Both *cis* and *trans* lactonized helenanolides are known and helenalin belong to the former group. The structure of helenalin has been determined by chemical and spectroscopic methods[2] and confirmed by X-ray diffraction studies of its 3-bromo derivative[3]. A search through the crystallographic literature has revealed that two polymorphic forms of native helenaline are known already, and their crystal structures have been published[4,5]. Both polymorphs crystallize in the same monoclinic space group $P2_1$ and exhibit the same molecular conformation. However their hydrogen bonding is different, as it involves different oxygen atoms as acceptors (carbonyl O(4) vs lactone carbonyl O(12). During the crystallization process carried out in our laboratory, we have obtained two polymorphic forms of helenalin, one identical with the monoclinic form already published by Fronczek et al.[4], and a new orthorhombic modification. We have decided to carry out an X-ray analysis for both types of crystals, although only orthorhombic form presents novel results, and to complement the investigations by the ^{13}C NMR study both in the solid state and solution.



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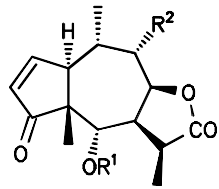

II, $R^1 = R^2 = H$

III, $R^1 = COC(CH_3)=CH_2$; $R^2 = OH$

RESULTS AND DISCUSSION

X-Ray Structure Analysis

Final positional parameters and U_{eq} for non-H atoms for the orthorhombic crystalline form of helenalin are given in Table I; bond distances, bond angles, and selected torsion angles observed in all three polymorphs are compared in Tables II – IV. The helenalin molecule is shown in Fig. 1 (ref.[6]). The ring junctions of the pseudoguaianolide nucleus are C(1)β,C(5)α *trans* and C(7)β,C(8)β *cis*. The conformation of the helenalin molecule in the three crystalline environments is almost the same and similar to the conformation found in the crystal structure of 3-bromohelenalin[3]. In all molecules the seven-membered ring adopts a deformed twist-chair conformation with a pseudo diad axis passing through C(10) and the midpoint of the C(6)–C(7) bond. The deviations from the ideal *TC*(10) form are more pronounced in monoclinic form *Ia* and 3-bromohelenalin, and less severe in orthorhombic form *Ib*. As a measure of the devia-

tions from the ideal twist chair form the sum (Σ) of the absolute values of differences of symmetry related torsion angles is used[7]. The Σ value is 44° for *Ia*, 27° for *Ib*, 37° for *Ic* and 40° for 3-bromohelenalin and may be compared with the ideal twist-chair value of 0°. The observed *TC*(10) conformation is significantly different from those reported to date for all other *cis*-lactonized helenanolides for which two main conformations have been observed: the twist-boat conformation with an approximate twofold axis passing through C(10) and the midpoint of the C(6)–C(7) bond [*TB*(10)], and the twist-chair conformation with an approximate twofold axis passing through C(7) and the midpoint of the C(1)–C(10) bond [*TC*(7)] (refs[8,9]).

The γ-lactone shows a puckered conformation, of the half-chair type, with C(7) above and C(8) below the plane through C(11), C(12) and O(8). The average torsion angle moduli is 22.2(9.2), 23.8(9.8) and 25.8(10.7)° for *Ia*, *Ib*, and *Ic*, respectively. The C(7)–H(7) and C(8)–O(8) bonds are synclinal giving rise to a positive torsion angle at the junction with the homocycle (O(8)–C(8)–C(7)–C(11)) while in *cis*-lactonized helenanolides studied previously this torsion angle was either negative or close to zero. If we use the notation proposed by Samek[10] the investigated compound has conformational type *S* while all previous examples were either *A* or *P*(A), respectively. For *cis*-

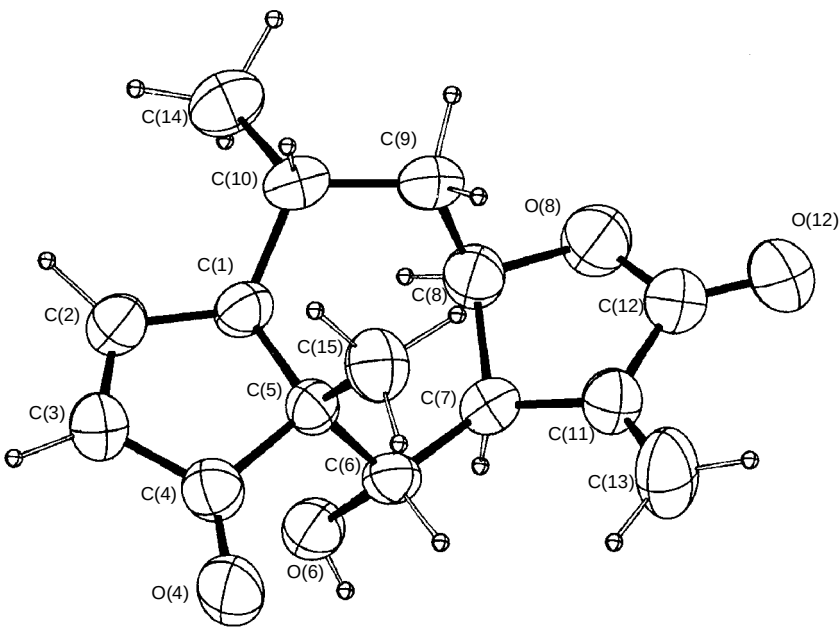

FIG. 1
View of the β-face of the molecule of the orthorhombic form (*Ib*) of helenalin and atom numbering scheme

lactonized helenanolides an *S*-type requires the C(7)–C(11) lactone bond axial and C(8)–O(8) bond equatorial, an unlikely situation according to Samek's generalization[10].

The α,β-unsaturated cyclopentenone shows significant ring puckering (the average torsion angle amplitude 11.2(4.7), 12.9(5.9) and 12.7(5.8)° for *Ia*, *Ib*, and *Ic*, respectively). In *Ia* the ring adopts a twist form with atoms at the ring-fusion sticking out of the best plane through the ring, while in *Ib* and *Ic* the envelope form predominates, with C(5) out of the C(1)…C(4) plane. Within the cyclopentenone ring the differences between the corresponding C(2)–C(3), C(3)–C(4) and C(4)–O(4) bond distances are greater than 3σ and such that they might be ascribed to the higher degree of electron delocalization in the conjugated –C=C–C=O system in *Ia* as compared with more localized bonds in *Ib* and *Ic*. More pronounced electron delocalization in *Ia* might in turn

TABLE I

Positional parameters (. 10^4) and equivalent isotropic thermal parameters (Å^2 . 10^3) with e.s.d.'s in parentheses for non-H atoms of helenalin orthorhombic form (*Ib*). $U_{eq} = 1/3 \sum_i \sum_j a_i^* a_j^* \mathbf{a}_i \mathbf{a}_j$

Atom	*x*/*a*	*y*/*b*	*z*/*c*	U_{eq}
C(1)	–0.2426(4)	0.3914(4)	0.8492(1)	56(1)
C(2)	–0.3780(5)	0.2556(6)	0.8582(2)	76(2)
C(3)	–0.3275(5)	0.1289(6)	0.8926(2)	82(1)
C(4)	–0.1528(5)	0.1626(5)	0.9149(1)	69(1)
C(5)	–0.1102(4)	0.3486(4)	0.8987(1)	53(1)
C(6)	0.0736(4)	0.3603(4)	0.8762(1)	51(1)
C(7)	0.1296(4)	0.5334(4)	0.8504(1)	49(1)
C(8)	0.0053(4)	0.6513(4)	0.8151(1)	57(1)
C(9)	–0.1593(4)	0.7051(4)	0.8454(2)	64(1)
C(10)	–0.3067(4)	0.5745(5)	0.8441(1)	64(1)
C(11)	0.2153(4)	0.6597(4)	0.8913(1)	57(1)
C(12)	0.2106(5)	0.8235(5)	0.8586(2)	64(1)
C(13)	0.2907(6)	0.6441(7)	0.9436(2)	87(2)
C(14)	–0.4136(5)	0.5987(6)	0.7869(2)	82(2)
C(15)	–0.1373(5)	0.4468(5)	0.9588(1)	71(1)
O(4)	–0.0604(4)	0.0650(4)	0.9420(1)	90(1)
O(6)	0.0943(3)	0.2398(3)	0.8277(1)	64(1)
O(8)	0.1078(3)	0.8095(3)	0.8100(1)	71(1)
O(12)	0.2878(3)	0.9540(3)	0.8692(1)	80(1)

be ascribed to the involvement of the carbonyl oxygen at C(4) in the hydrogen bond formation with C(6) hydroxyl group, a situation that is not observed in *Ib* and *Ic*.

The packing of helenalin molecules in the three crystalline forms is governed by O–H…O hydrogen bonding in which the O(6) hydroxyl group always acts as a single proton donor, but the role of an acceptor is taken over by different carbonyl groups. In *Ia* the hydroxyl group at C(6) donates its proton to the screw-axis-related carbonyl oxygen at C(4) (vide supra) while in *Ib* and *Ic* it forms hydrogen bonds with the lactone carbonyl oxygen (related by a single translation along the *y* direction in *Ib* and by screw axis in *Ic*). The packing of helenalin molecules in the three crystal structures is com-

TABLE II
Bond lengths (Å) observed in three polymorphic forms *Ia* – *Ic* of helenalin

Atoms	*Ia*[a]	*Ib*[b]	*Ic*[c]
C(1)–C(2)	1.508(6)	1.515(5)	1.505(3)
C(1)–C(5)	1.552(4)	1.545(4)	1.546(3)
C(1)–C(10)	1.531(4)	1.526(5)	1.530(3)
C(2)–C(3)	1.338(4)	1.313(6)	1.323(4)
C(3)–C(4)	1.457(5)	1.476(6)	1.456(3)
C(4)–O(4)	1.225(3)	1.212(5)	1.211(3)
C(4)–C(5)	1.528(4)	1.539(5)	1.537(3)
C(5)–C(15)	1.549(4)	1.553(4)	1.529(3)
C(5)–C(6)	1.517(5)	1.523(4)	1.529(3)
C(6)–C(7)	1.549(5)	1.537(4)	1.532(3)
C(6)–O(6)	1.437(3)	1.440(4)	1.422(3)
C(7)–C(8)	1.536(4)	1.553(4)	1.543(3)
C(7)–C(11)	1.503(5)	1.501(4)	1.505(3)
C(8)–C(9)	1.532(5)	1.511(5)	1.516(3)
C(8)–O(8)	1.468(4)	1.482(4)	1.491(2)
C(9)–C(10)	1.548(6)	1.542(5)	1.540(3)
C(10)–C(14)	1.546(5)	1.529(5)	1.525(3)
C(11)–C(12)	1.485(4)	1.476(5)	1.482(3)
C(11)–C(13)	1.313(5)	1.305(5)	1.312(3)
C(12)–O(12)	1.209(5)	1.212(5)	1.210(3)
C(12)–O(8)	1.338(5)	1.348(5)	1.339(2)

[a] Structure solved by Fronczek et al.[4]; geometrical parameters calculated from our X-ray results. [b] Present X-ray study. [c] Data taken from ref.[5].

TABLE III
Bond angles (°) observed in three polymorphic forms *Ia* – *Ic* of helenalin

Atoms	*Ia*[a]	*Ib*[b]	*Ic*[c]
C(2)–C(1)–C(5)	103.1(3)	102.8(3)	103.2(2)
C(2)–C(1)–C(10)	117.4(3)	116.3(3)	116.3(2)
C(5)–C(1)–C(10)	117.3(2)	118.5(2)	117.8(2)
C(1)–C(2)–C(3)	113.1(3)	113.5(3)	112.9(2)
C(2)–C(3)–C(4)	109.3(3)	109.7(4)	110.2(2)
C(3)–C(4)–O(4)	128.0(3)	127.2(4)	127.9(2)
C(5)–C(4)–O(4)	123.6(3)	125.9(3)	124.9(2)
C(3)–C(4)–C(5)	108.4(2)	107.0(3)	107.1(2)
C(4)–C(5)–C(15)	104.0(2)	104.0(2)	104.2(2)
C(6)–C(5)–C(15)	113.1(3)	112.3(3)	113.2(2)
C(1)–C(5)–C(4)	103.2(2)	103.1(2)	103.0(1)
C(1)–C(5)–C(6)	110.4(2)	112.7(2)	111.4(2)
C(4)–C(5)–C(6)	109.1(2)	109.7(3)	109.6(2)
C(1)–C(5)–C(15)	116.1(2)	114.2(3)	114.6(2)
C(5)–C(6)–O(6)	105.2(3)	108.1(2)	106.1(1)
C(5)–C(6)–C(7)	116.8(3)	116.3(3)	116.0(2)
C(7)–C(6)–O(6)	108.1(2)	105.7(2)	110.7(2)
C(6)–C(7)–C(8)	122.5(3)	122.4(3)	121.1(2)
C(6)–C(7)–C(11)	117.8(2)	119.1(2)	120.0(2)
C(8)–C(7)–C(11)	101.6(2)	100.9(2)	100.6(2)
C(7)–C(8)–C(9)	119.9(3)	118.4(2)	118.2(2)
C(7)–C(8)–O(8)	102.0(2)	101.5(2)	100.7(1)
C(9)–C(8)–O(8)	105.7(3)	105.1(2)	104.6(2)
C(8)–C(9)–C(10)	117.1(3)	116.2(3)	117.7(2)
C(1)–C(10)–C(9)	111.9(3)	112.3(3)	111.9(2)
C(1)–C(10)–C(14)	109.4(3)	111.0(3)	109.4(2)
C(9)–C(10)–C(14)	110.7(3)	109.9(3)	111.2(2)
C(7)–C(11)–C(12)	105.6(3)	105.5(3)	104.3(2)
C(7)–C(11)–C(13)	132.3(3)	132.5(3)	133.1(2)
C(12)–C(11)–C(13)	121.8(3)	121.9(4)	122.6(2)
C(11)–C(12)–O(8)	108.6(3)	109.6(3)	109.7(2)
C(11)–C(12)–O(12)	129.3(3)	129.0(4)	129.2(2)
O(8)–C(12)–O(12)	122.0(3)	121.4(4)	121.2(2)
C(8)–O(8)–C(12)	110.9(2)	109.3(3)	109.2(1)

[a] Structure solved by Fronczek et al.[4]; geometrical parameters calculated from our X-ray results. [b] Present X-ray study. [c] data taken from ref.[5].

pared in Figs 2 to 4, and the intermolecular hydrogen bond parameters are listed in Table V. Forms *Ia* and *Ib* crystallize in more open lattices than form *Ic*. In the monoclinic forms *Ia* and *Ic*, Figs 2 and 4, respectively, the hydrogen bonded molecules are related by the *y* screw axis and form infinite helical chains running along this axis. We note, however, that different hydrogen-bond acceptors are involved in the bonding (O(4) in *Ia*, and O(12) in *Ic*). In the orthorhombic form *Ib* (Fig. 3), the hydrogen bonded molecules are related by a single translation along *y*-axis. As in *Ic*, the O(12) oxygen atom is the acceptor in the hydrogen bond with the O(6) hydroxyl group.

Owing to the scarcity of hydrogen-bond donors with respect to acceptors, the packing is completed by the C–H…O bonds (Table V). The C–H groups involved (C(13) in *Ib*; C(2) and C(8) in *Ic*) are close to the electronegative atoms that make them presumably more acidic and, consequently, good candidates to be involved in CH…O hydrogen bonds[11]. The molecules thus connected form double molecular layers in *Ib* or extended in all three directions in a more densely packed structure *Ic*.

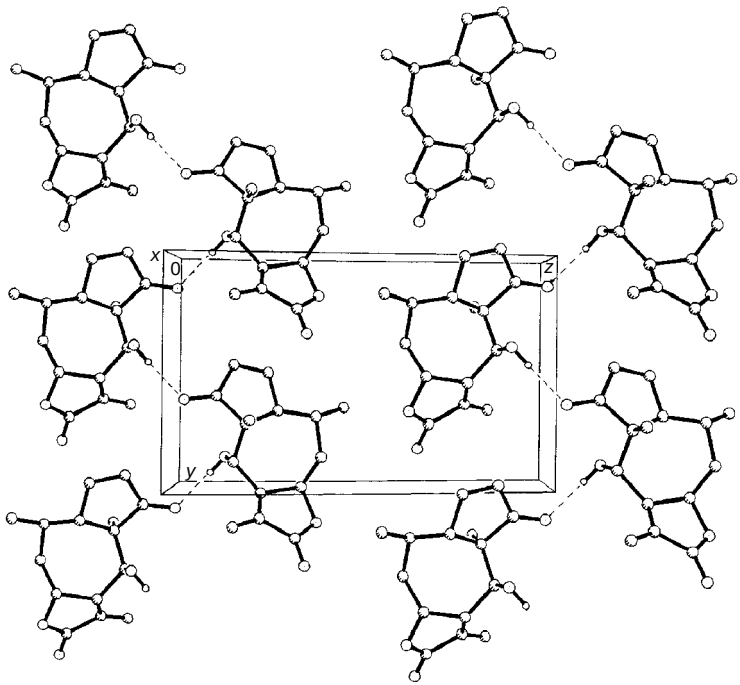

FIG. 2
Unit cell packing of the monoclinic form (*Ia*) of helenalin viewed along the *x*-axis. Molecules related by the screw axis are linked by the O(6)–H…O(4) hydrogen bond. H-atoms not involved in H-bonding have been omitted for clarity

NMR Study

Two main goals of this NMR study were: (i) to determine the conformational behaviour of helenalin (*I*) in solution as compared with the solid state conformation, (ii) to obtain the solid-state ^{13}C NMR data of two crystalline forms of helenalin (*Ia*) and (*Ib*) and to correlate the chemical shift data with the different hydrogen-bonding patterns detected by X-ray structure analysis.

*NMR Study of Helenalin (*I) in Solution

Basic sets of ^{1}H and ^{13}C NMR data on helenalin (*I*) in $CDCl_3$ were described in one of our previous papers[12] and ^{13}C NMR data were published also by other authors[13]. However, the detailed interpretation of the NMR parameters in the sense of conformational behaviour has not yet been done and is a subject of this paper. Main attention has been payed to the seven-membered homocycle, the conformation of which defines the shape of *cis*-annellated lactone ring and the configuration at C(1) and C(5) determines the shape of *trans*-annellated cyclopentenone.

To estimate a possible influence of solvent on the conformation of helenalin (*I*) we have measured the ^{1}H and ^{13}C NMR spectra in a series of solvents. The extremely low solubility in cyclohexane did not allow to collect ^{13}C data in this solvent. The structural assignment of most of the proton signals on the basis of the chemical shifts and the topology of interproton couplings is straightforward. The stereochemical assignment of geminal protons in position 9 and 13, suggested earlier on the basis of vicinal coupling values (at C(9)) and homoallylic couplings and lactone carbonyl deshielding effect (at C(13)), has been confirmed by us by 2D-ROESY spectra (only 9β-H shows ROESY crosspeak with β-oriented methyl-15 protons and only upfield shifted H-13′ (c.f.*) gives ROESY crosspeaks to H-6 and H-7). Proton NMR parameters in seven solvents are summarized in Table VI. The one-bond correlated ^{1}H-^{13}C HMQC and longe-range HMBC experiment (adjusted for J(C,H) ca 7 Hz) in $CDCl_3$ has confirmed our previous assignment[12] of all carbon signals. ^{13}C NMR signals in other solvents could be assigned by analogy from the corresponding "attached proton test" spectra. Carbon-13 chemical shifts of helenalin (*I*) in five different solvents are given in Table VII. Relaxation times T_1 of carbon atoms were measured in $CDCl_3$. The obtained data (Table VII) show nearly identical NT_1 values for all protonated ring carbon atoms ($NT_1 \approx 1.4$ s; N number of protons bonded to given carbon atom) and substantially longer T_1's (6.4 – 15 s) for quaternary carbons C(4), C(5), C(11) and C(12).

* Proton H-13′ is *trans* oriented to lactone carbonyl while H-13 is *cis* oriented.

The chemical shifts of individual protons as well as carbon atoms vary in rather large range within a given set of solvents (differences from –0.65 to +0.54 ppm for protons and from –1.44 ppm to +2.56 ppm for carbon atoms, relative to the value in cyclohexane for ^{1}H and chloroform for ^{13}C). Remarkable differences were observed even between proton shifts in chloroform and cyclohexane (all positive between 0.06 and 0.26 ppm). On the other hand the interproton coupling constants show very little changes with solvent and the only significant differences (0.5 – 1.0 Hz) were detected for $J(8,9\beta)$, $J(9\alpha,10)$ and $J(9\beta,10)$ values between nonpolar and polar solvents. We believe that even such differences do not indicate remarkable conformational change and allow us to expect a similar conformational behaviour of helenalin in all solvents used.

Correlation between interproton dihedral angles found in polymorphs *Ia*, *Ib* and vicinal coupling constants observed in solution (see Table VIII) shows in principle a good semiquantitative agreement for all *J*'s except for $J(8,9\alpha)$, $J(8,9\beta)$ and, especially,

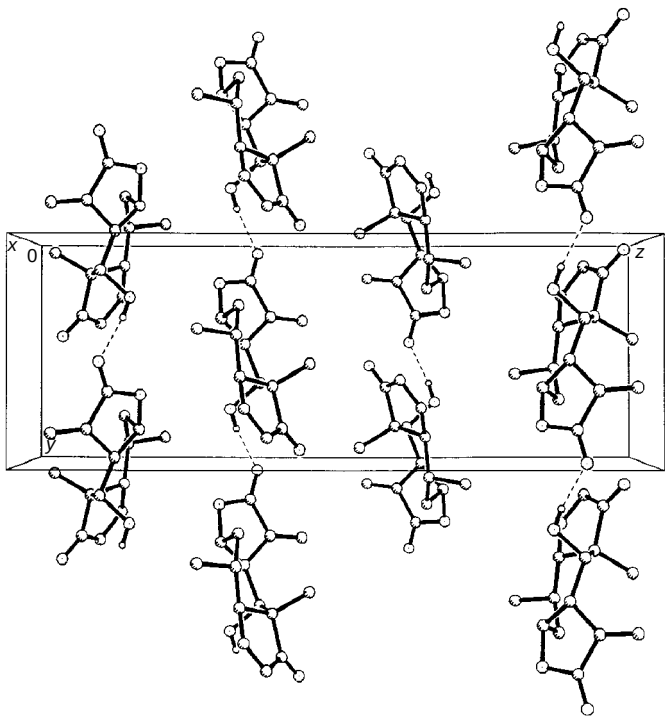

FIG. 3
Unit cell packing of the orthorhombic form (*Ib*) of helenalin viewed along the *x*-axis. Molecules related by a single translation along *y*-axis are linked by the O(6)–H…O(12) hydrogen bond. H-atoms not involved in H-bonding have been omitted for clarity

J(9α,10), *J*(9β,10). From the inspection of models we were not able to find any single conformation allowing to explain the observed set of *J*-values. The presence of two conformers of helenalin in solution was already suggested by McPhail and Onan[14] to explain the absence of an observable Cotton effect for $n \rightarrow \pi^*$ transition of the α-methylene-γ-lactone chromophore. The positive chirality, resulting from a positive torsion angle around C(7)–C(8) bond in crystal conformation *TC*(10) of helenalin (*I*), can be compensated by the presence of a conformer with a negative chirality (negative torsion angle around C(7)–C(8)). Such conformers of the type *TC*(7) were found for structurally very similar compounds – e.g. plenolin[14] (*II*) and radiatin[15] (*III*) – in crystal. It may indicates a similar energy of both conformers – *TC*(10) and *TC*(7) – and their facile interconversion in solution[14]. The calculated torsion angles of protons and corresponding vicinal coupling constants in seven-membered ring of *TC*(7) conformer, represented by crystal data on plenolin[14] (*II*), are also given in Table VIII. The comparison of molecular conformations of helenalin (*I*) and plenolin (*II*) in crystal is given in Fig. 5. It can be shown that the presence of second ("plenolin-type") conformer *TC*(7) improves an agreement between calculated and observed *J*-values dramatically (especially *J*(8,9α), *J*(8,9β), *J*(9α,10), *J*(9β,10)) with a best fit found for ca 1 : 1 ratio (Table VIII). Small changes of that ratio can be responsible for above mentioned differences of *J*-values observed in non-polar and polar solvents. Low-temperature ^{1}H NMR spectra of hele-

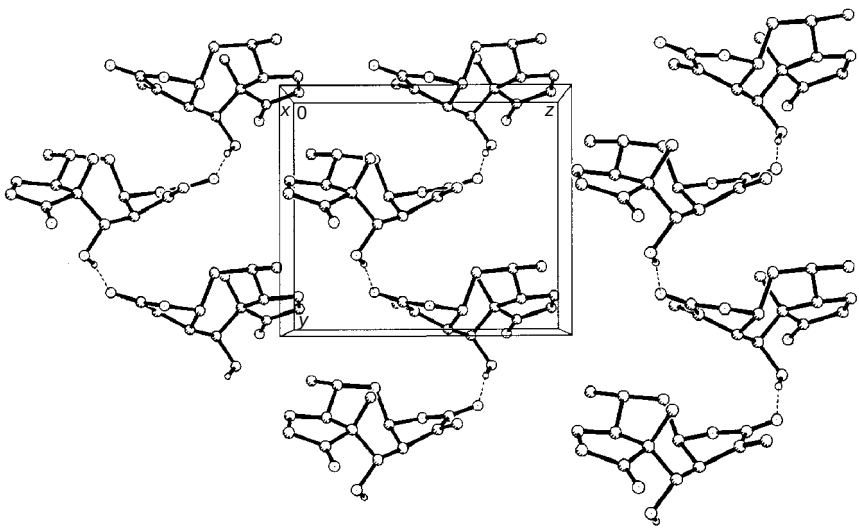

FIG. 4
Unit cell packing of the monoclinic form (*Ic*) of helenalin viewed along the *x*-axis. Molecules related by the screw axis are linked by the O(6)–H…O(12) hydrogen bond. H-atoms not involved in H-bonding have been omitted for clarity

nalin (*I*) in CD_2Cl_2 (in the range 0 °C to –60 °C) did not show substantial line-broadening or even two set of signals. Conformation equilibrium in helenalin solution is probably still too fast on NMR time scale.

Table IX presents the results of 2D-ROESY experiment on helenalin (*I*) in $CDCl_3$ together with the interproton distances calculated for *TC*(10) and *TC*(7) conformers from the X-ray data of helenalin (*I*) and plenolin (*II*) in crystal. ROESY crosspeaks have been found in general for pairs of protons with internuclear distance shorter than around 3.5 Å in both and/or at least one crystal conformations (in accordance with a common borderline for a small molecules). Relative intensities of crosspeaks well reflect calculated distances showing strong peaks for a distance range approximately < 2.7 Å and weak peaks for more distant protons. The observation of ROESY crosspeaks between protons which are either sufficiently close only in the *TC*(7) conformer (e.g. H(9β)/H(14)) and/or those which intensities correspond to the shorter distance in *TC*(7) (e.g. H(1)/H(9α), H(8)/H(9β), H(2)/H(14), H(10)/H(15)) provide the additional arguments for the *TC*(10) ↔ *TC*(7) conformational equilibrium in solution.

It is well known that the additional source of structural information can be obtained from proton-carbon coupling constants (for review see ref.[17]). Technical difficulties connected with obtaining the experimental values of geminal and vicinal *J*(C,H) have

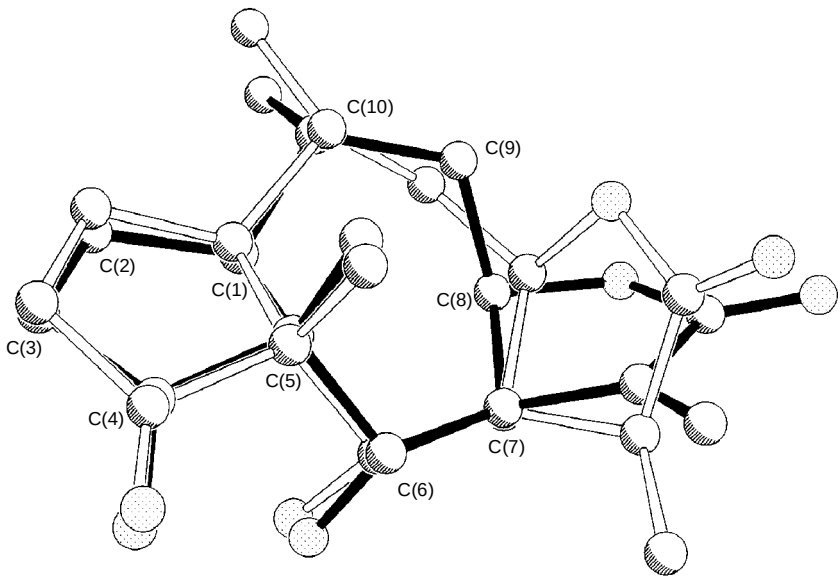

FIG. 5
The comparison of molecular conformation of helenalin (*I*) (dark lines) and plenolin (*II*) (open lines) in crystal

limited their stereochemical utilization to either very simple molecules or specifically ^{13}C labelled compounds. Distribution of proton signals in 500 MHz ^{1}H NMR spectrum (allowing a sufficiently selective proton decouplings required for individual *J*(C,H) assignment) motivated us to attempt to collect extensive set of *J*(C,H) couplings for helenalin (*I*). The presence of long-range *J*(C,H)'s was proven by 2D HMBC experiment and individual values were derived from comparison of a proton-coupled ^{13}C NMR spectra with a complete set of selectively proton decoupled spectra. Table X defines all *J*(C,H) over one, two and three bonds in helenalin molecule and summarizes the ob-

TABLE IV
Endocyclic torsion angles (°) observed in three polymorphic forms *Ia* – *Ic* of helenalin

Atoms	*Ia*[a]	*Ib*[b]	*Ic*[c]
	5-Membered ring		
C(5)–C(1)–C(2)–C(3)	−13.2(4)	−14.4(4)	−14.1(3)
C(2)–C(1)–C(5)–C(4)	16.3(3)	18.8(3)	18.5(2)
C(1)–C(2)–C(3)–C(4)	3.9(4)	2.8(5)	2.7(3)
C(2)–C(3)–C(4)–C(5)	7.6(4)	10.3(5)	10.1(3)
C(3)–C(4)–C(5)–C(1)	−15.1(3)	−18.3(3)	−17.9(2)
	7-Membered ring		
C(10)–C(1)–C(5)–C(6)	−96.6(3)	−93.1(3)	−94.6(2)
C(5)–C(1)–C(10)–C(9)	48.5(4)	47.4(4)	50.9(2)
C(1)–C(5)–C(6)–C(7)	61.1(3)	58.1(3)	61.6(2)
C(5)–C(6)–C(7)–C(8)	−33.1(4)	−34.1(4)	−40.5(3)
C(6)–C(7)–C(8)–C(9)	50.8(4)	56.0(4)	60.2(3)
C(7)–C(8)–C(9)–C(10)	−77.1(4)	−80.6(4)	−79.2(2)
C(8)–C(9)–C(10)–C(1)	34.5(4)	35.3(4)	30.9(2)
	γ-Lactone ring		
O(8)–C(8)–C(7)–C(11)	32.7(3)	35.2(3)	38.3(2)
C(7)–C(8)–O(8)–C(12)	−29.2(3)	−31.1(3)	−32.2(2)
C(8)–C(7)–C(11)–C(12)	−26.5(3)	−28.4(3)	−32.4(2)
C(7)–C(11)–C(12)–O(8)	9.8(4)	10.6(4)	14.0(2)
C(11)–C(12)–O(8)–C(8)	12.7(4)	13.6(4)	12.0(3)

[a] Structure solved by Fronczek et al.[4]; geometrical parameters calculated from our X-ray results. [b] Present X-ray study. [c] Data taken from ref.[5].

tained *J*(C,H) values. For vicinal *J*(C,H) the corresponding carbon-proton dihedral angles in *TC*(10) and *TC*(7) conformer (calculated form X-ray data of helenalin (*I*) and plenolin (*II*) are also given. The ^1J(C,H) values clearly manifest the effect of hybridization and substitution with electronegative oxygen (J = 160 – 174 Hz for sp^2-carbon atoms C(2), C(3) and C(13); J = 144.4 – 151.4 Hz for oxygen bearing C(6) and C(8) carbons; J = 126 – 131 Hz for all other carbon present). Unfortunately not all geminal and vicinal *J*(C,H) could be determined. Large number of these *J*(C,H)'s with contribution of some non-zero long-range *J*(C,H)'s leads to poorly resolved multiplets for some carbon atoms (C(1), C(5), C(6), C(7) and C(9)) as it is illustrated in Fig. 6. The largest vicinal *J*(C,H) ≈ 13 Hz appear for carbonyl C(4) and C(12) atoms *trans*-oriented to hydrogen in H–C=C–C=O fragment (corresponding value of *cis*-coupling for C(12) is 6.8 Hz). Karplus-like relation between dihedral angle and ^3J(C,H), similar as for ^3J(H,H), was supported with both theoretical and empirical data[17] (the average ratio ^3J(C,H)/^3J(H,H) ≈ 0.6 was found for couplings in geometry equivalent systems). The *J*(C,H) values observed for helenalin agree in general with the existence of two conformers – *TC*(10) and *TC*(7) – in solution: in the case of similar dihedral angle in both forms the observed *J*(C,H) corresponds to the expected value for given angle (e.g. *J*(C4,H1) ≈ 0

TABLE V
Hydrogen-bond parameters for three polymorphic forms *Ia* – *Ic* of helenalin

Compound	D–H...A acceptor position	D–H, Å	H...A, Å	D...A, Å	D–H...A, °
Ia[a]	O(6)–H...O(4)	0.83(4)	1.91(4)	2.728(4)	173(1)
	–*x*, 1/2 + *y*, –*z*				
Ib[b]	O(6)–H...O(12)	0.83(4)	2.04(4)	2.857(4)	171(1)
	x, *y* – 1, *z*				
	C(13)–H...O(4)	0.99(4)	2.46(4)	3.235(4)	134(1)
	1/2 + *x*, 1/2 – *y*, 2 – *z*				
Ic[c]	O(6)–H...O(12)	0.85	2.02	2.859(3)	171
	–*x*, 1/2 + *y*, 1 – *z*				
	C(2)–H...O(4)	0.96	2.55	3.243(3)	130
	1 – *x*, *y* – 1/2, –*z*				
	C(8)–H...O(4)	0.96	2.50	3.376(3)	152
	x – 1, *y*, *z*				

[a] Structure solved by Fronczek et al.[4]; geometrical parameters calculated from our X-ray results. [b] Present X-ray study. [c] Calculated from atomic coordinates taken from ref.[5].

Hz for angles close to −90°) while in the case of significantly different dihedral angles the observed *J*(C,H) usually fits better an average value which can result from the individual conformers (e.g. *J*(C12,H7) ≈ *J*(C12,H8) ≈ 2.8 Hz for calculated angles 92°, 144° and −149°, −89° Hz in *TC*(10), *TC*(7) forms of helenalin (see Table X).

Solid State NMR Spectra

High resolution solid state CP-MAS ^{13}C NMR spectra were recognized to be the conformation dependent (for review see e.g. ref.[18]). Complementary X-ray and solid state

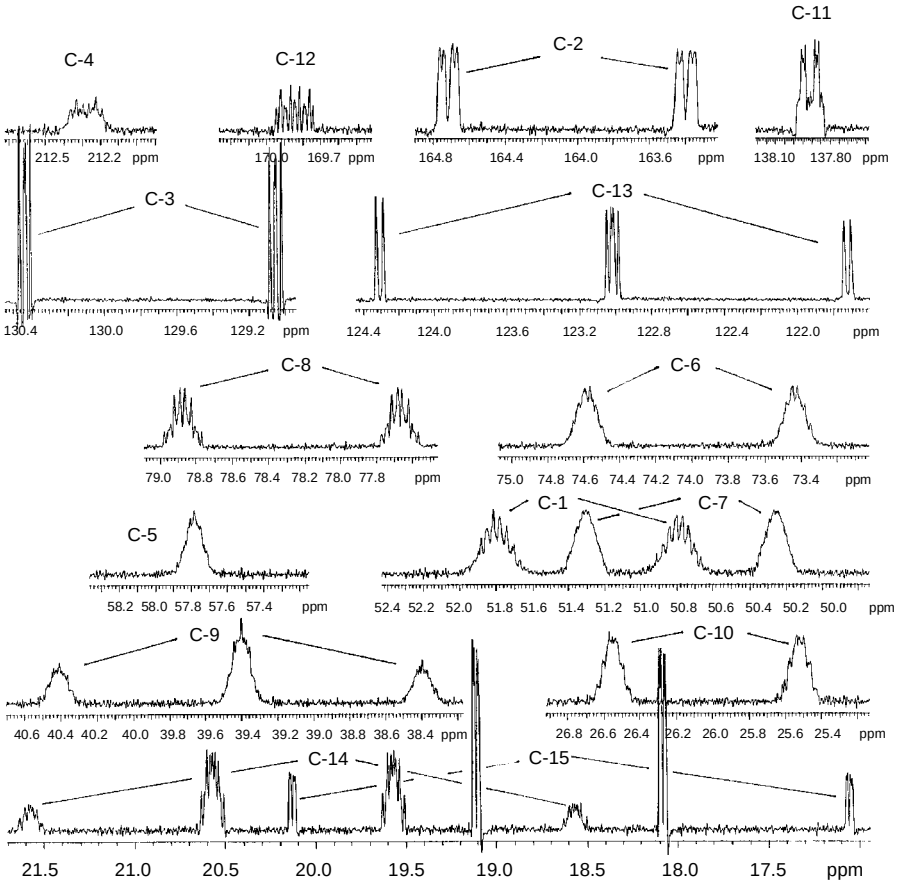

FIG. 6
Proton-coupled ^{13}C NMR spectrum of helenalin (*I*) in $CDCl_3$

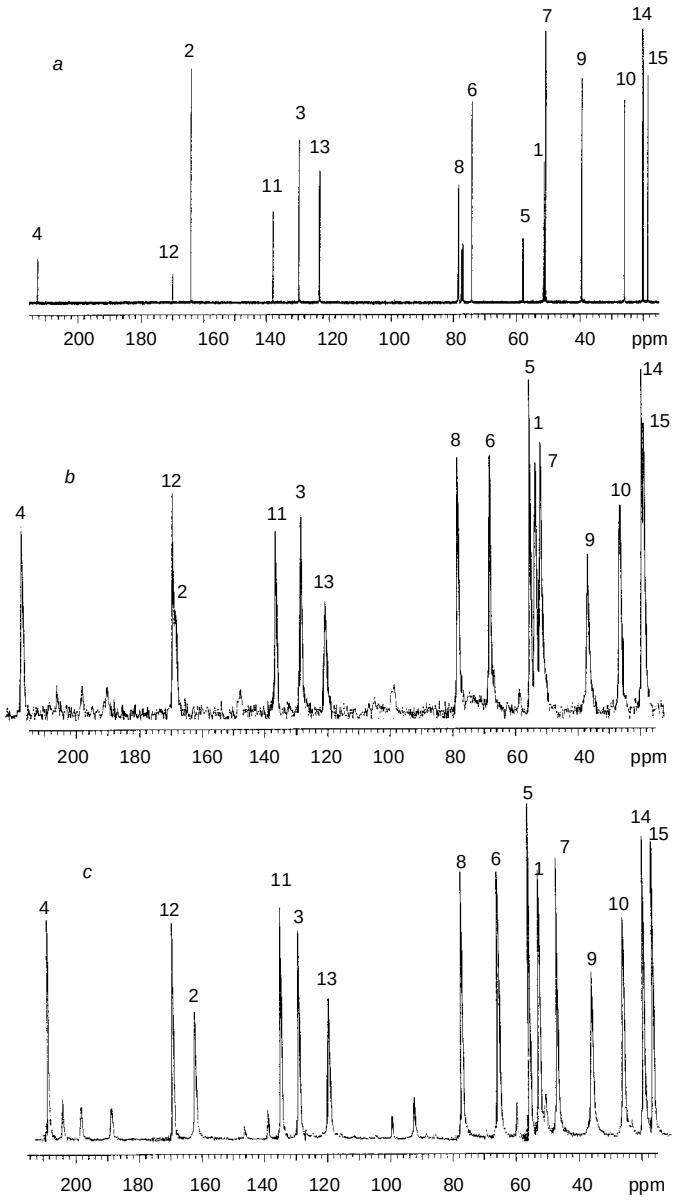

FIG. 7
^{13}C NMR spectra of helenalin (*I*): *a* in $CDCl_3$ solution; *b* and *c* solid state CP-MAS spectra of monoclinic (*Ia*) and orthorhombic form (*Ib*), respectively

TABLE VI
Proton NMR parameters of helenalin (*I*) in different solvents

Parameter	$CDCl_3$[a]	CD_2Cl_2[b]	C_6D_{12}	C_6D_6	CD_3OD	C_5D_5N	CD_3COCD_3
Proton				chemical shifts, ppm			
H-1	3.08 ddd	3.05	3.02	2.75	3.18	3.48	3.21
H-2	7.69 dd	7.71	7.47	6.82	7.83	7.58	7.78
H-3	6.08 dd	6.06	5.90	5.71	6.02	6.14	5.96
H-6	4.47 bdd	4.45	4.34	4.23	4.35	4.84	4.39
OH-6	2.63 d	2.45	[c]	[c]	[c]	[c]	4.56
H-7	3.56 ddt	3.55	3.43	3.01	3.53	3.87	3.59
H-8	4.98 ddd	4.98	4.72	4.33	5.03	5.14	5.03
H-9α	1.82 ddd	1.82	1.72	1.30	1.81	1.83	1.80
H-9β	2.27 ddd	2.25	2.17	1.75	2.29	2.22	2.26
H-10	2.07 m	2.06	2.00	1.46	2.06	1.94	2.05
H-13	6.38 d	6.32	6.20	6.21	6.28	6.34	6.20
H-13′	5.80 d	5.80	5.54	5.21	5.85	5.63	5.81
H-14	1.27 d	1.28	1.21	0.63	1.27	1.07	1.27
H-15	1.00 s	1.00	0.93	0.73	0.90	1.02	0.88
H*i*,H*j*				coupling constants, Hz			
1,2	1.9	1.9	1.9	1.9	2.0	1.9	2.0
1,3	2.9	2.9	3.0	2.9	2.9	3.1	3.0
1,10	11.6	11.8	11.7	11.7	11.5	11.4	11.4
2,3	6.0	5.9	6.0	5.9	6.0	5.9	6.0
6,OH	4.4	4.2	[c]	[c]	[c]	[c]	4.4
6,7	1.9	2.1	1.9	2.1	1.7	1.8	1.7
7,8	7.7	7.5	7.8	7.7	7.8	7.6	7.8
7,13	3.1	3.2	3.0	3.1	2.9	3.1	2.9
7,13′	2.8	2.9	2.7	2.8	2.9	2.8	2.7
8,9α	2.5	2.6	2.4	2.5	2.4	2.5	2.7
8,9β	8.7	9.1	8.7	8.6	7.8	8.0	8.0
9α,9β	14.7	15.0	14.7	14.9	14.9	14.9	14.9
9α,10	6.2	6.1	6.3	6.5	7.6	7.4	7.3
9β,10	4.5	4.9	4.5	4.5	3.9	4.0	3.9
10,14	6.7	6.6	6.6	6.7	6.6	6.6	6.6

[a] The same signal multiplicities as in $CDCl_3$ are observed in other given solvents. [b] The additional $J(2,10) = 0.6$ Hz was observed. [c] The value of parameter could not be determined.

NMR study of some crystalline polymorphs demonstrated the sensitivity of ^{13}C chemical shifts to molecular conformation. With our crystalline polymorphs of helenalin (*Ia*, *Ib*) we met a rather unique situation when polymorphs have nearly identical conformation and they differ mainly in hydrogen bonding network. Their solid state NMR spectra should therefore reflect only such a delicate difference.

Both polymorphs *Ia* and *Ib* provided well resolved solid state CP-MAS ^{13}C NMR spectra (Fig. 7) with chemical shift differences up to 6.30 ppm for carbon atom at a given position in *Ia* and *Ib* (see Table XI). Type of H-bonding is well reflected by shift differences of carbon atoms of the C=O and C–OH groups involved in H-bond formation. Very small difference (0.64 ppm) is observed for C(6)–OH in accordance with its function of proton donor in both *Ia* and *Ib* form while large differences are found for C(4) and C(12) carbonyl carbons (6.30 and 2.36 ppm) operating as proton acceptors in *Ia* and *Ib*, respectively, and for C(3) and C(2) carbons in α- and β-position of unsaturated ketone (4.08 and 2.88 ppm).

TABLE VII
Carbon-13 relaxation times T_1 and chemical shifts of helenalin (*I*) in different solvents

Carbon	T_1, s $CDCl_3$	Chemical shifts, ppm				
		$CDCl_3$	C_6D_6	CD_3OD	C_5D_5N	CD_3COCD_3
C-1	1.43	51.34	51.18	53.04	52.44	52.34
C-2	1.13	163.88	162.88	165.88	164.00	163.85
C-3	1.12	129.99	129.68	130.29	129.72	130.03
C-4	9.01	212.21	211.09	213.80	211.47	210.77
C-5	14.86	57.98	57.62	58.48	57.85	57.69
C-6	1.46	74.10	74.10	75.44	74.14	75.02
C-7	1.44	50.76	51.18	52.74	51.87	52.13
C-8	1.36	78.15	77.56	80.55	79.22	79.12
C-9	0.72	39.40	39.51	41.07	40.35	40.76
C-10	1.36	26.17	26.04	27.45	26.51	27.02
C-11	6.41	137.86	138.73	140.42	139.69	140.24
C-12	14.28	169.74	169.13	172.18	170.42	170.20
C-13	0.56	123.05	121.90	123.34	121.94	122.07
C-14	0.93	20.15	19.74	20.46	20.16	20.40
C-15	0.97	18.69	18.67	19.27	18.81	18.94

Two sets of solid state carbon-13 shifts for *Ia* and *Ib* differ also from chemical shifts in solution (see Table XI). Assuming the absence of intermolecular association of substrate in dilute solution in non-polar solvent ($CDCl_3$) and close conformational similarity in both phases, the observed differences $\Delta\delta_C = \delta_C(\text{solid}) - \delta_C(CDCl_3)$ can be interpreted as the shielding effects resulting from intermolecular interactions in crystal. For monoclinic form *Ia* the largest $\Delta\delta_C$ are observed for C-6 (–5.84 ppm), C-4 (5.61 ppm) and C-2 (5.16 ppm) in agreement with H-bonding between C(6)–OH) (proton donor; negative $\Delta\delta_C$) and C(4)=O (proton acceptor; positive $\Delta\delta_C$) and associated with it higher electron delocalization in the conjugated α,β-unsaturated ketone residue (vide supra). On the other hand the orthorhombic form *Ib* shows a similar large effect on C-6 (–6.48 ppm) but significant positive effect on C-12 (2.37 ppm) while C-4 stays nearly unaffected. Analogously it can be interpreted as the result of the intermolecular H-bonding between C(6)–OH (proton donor; negative $\Delta\delta_C$) and lactone carbonyl C(12)=O (acceptor; positive $\Delta\delta_C$).

The attempt to induce similar effect in solution ^{13}C NMR spectra of helenalin (*I*) was not fully successful. Two other solvents were used expecting they will form hydrogen bondings to keto and lactone carbonyl oxygen atoms (methanol) and to hydroxy group

TABLE VIII
Proton–proton dihedral angles in crystalline forms of helenalin (*I*) and plenolin (*II*), calculated and observed values of corresponding vicinal coupling constants

Protons	Dihedral angles, °		Interproton vicinal couplings, Hz			
			calculated for[c]:			observed for:
	I[a]	*II*[b]	*I*	*II*	*I* + *II* (1 : 1)[d]	*I*
H6–C6–C7–H7	96	99	0.5	0.8	0.7	1.9
H7–C7–C8–H8	39	–31	6.2	7.4	6.8	7.7
H8–C8–C9–H9α	–80	59	0.8	3.1	2.0	2.5
H8–C8–C9–H9β	161	–58	11.8	3.3	7.6	8.7
H9α–C9–C10–H10	–86	171	0.5	12.9	6.7	6.2
H9β–C9–C10–H10	32	–72	7.2	1.5	4.4	4.5
H10–C10–C1–H1	172	–162	12.9	12.0	12.4	11.6

[a] The average values calculated from X-ray data for polymorphs *Ia* and *Ib* presented. [b] Calculated from X-ray data taken from ref.[14]. [c] *J*(H,H) values calculated from dihedral angles in *I* and *II* using the Karplus-like equation from ref.[16]. [d] Calculated time averaged values for the equilibirum mixture of two helenalin conformers corresponding to the crystal conformers of helenalin (*I*) and plenolin (*II*).

TABLE IX

The observed NOE's in $CDCl_3$ solution of helenalin (*I*) and interproton distances calculated for *TC*(10) and *TC*(7) conformers from the X-ray data of helenalin (*I*) and plenolin (*II*)

	H-1	H-2	H-3	H-6	H-7	H-8	H-9α	H-9β	H-10	H-13	H-13′	H-14	H-15
						Calculated interproton distances, Å[a]							
H-1		2.76 *2.77*	4.00 *3.74*	3.60 *3.67*	3.16 *3.48*	2.06 *3.94*	3.42 *2.19*	3.36 *3.44*	2.86 *2.90*	6.10 *b*	5.24 *b*	2.87 *2.58*	3.94 *3.64*
H-2	++		2.54 *2.10*	5.42 *5.45*	5.80 *6.12*	4.66 *6.34*	4.99 *4.50*	4.88 *5.11*	3.00 *3.56*	8.16 *b*	7.16 *b*	3.23 *2.36*	4.53 *3.67*
H-3	+	++		5.00 *5.06*	6.22 *6.52*	6.02 *7.31*	6.73 *5.82*	6.02 *6.60*	4.61 *4.74*	8.27 *b*	6.88 *b*	5.54 *4.31*	4.46 *3.64*
H-6					2.40 *2.62*	3.94 *4.20*	5.12 *4.50*	3.85 *5.24*	4.74 *4.35*	3.66 *b*	2.12 *b*	6.06 *5.97*	2.92 *2.44*
H-7	+			++		2.34 *2.18*	4.28 *3.01*	3.78 *3.92*	5.00 *4.42*	3.98 *b*	3.28 *b*	5.39 *5.30*	4.48 *3.85*
H-8	++				++		2.50 *2.32*	2.94 *2.30*	3.64 *3.74*	5.26 *b*	4.93 *b*	3.24 *4.63*	4.66 *3.85*
H-9α	++				+	++		1.76 *1.71*	2.60 *2.94*	5.38 *b*	5.54 *b*	2.83 *2.51*	4.40 *4.01*
H-9β	+					++	++		2.26 *2.45*	4.14 *b*	4.08 *b*	3.81 *2.40*	2.83 *3.81*
H-10	++	+					++	++		6.16 *b*	5.69 *b*	2.65 *2.44*	3.05 *2.13*
H-13											1.76 *b*	7.51 *b*	3.69 *b*
H-13′				++	+					++		7.22 *b*	3.51 *b*
H-14	+	++				+	++	++	++				5.31 *3.96*
H-15		+	+	++				++	++		+		
						The observed interproton 2D-ROESY peaks in $CDCl_3$ solution[c]							

[a] The given interproton distances for helenalin (*I*) are the average values found in two forms *Ia* and *Ib* (differences are less than 0.2 Å); for methyl protons H-14 and H-15 the shortest value found is given; interproton distances for plenolin (*II*) were calculated from X-ray data taken from ref.[14] and are given in italics. [b] Plenolin (*II*) contains a methyl group instead of exomethylene protons H-13, H-13′. [c] 2D-ROESY cross peaks are indicated with a plus signs: (++) corresponds to a strong and (+) to a weak intensity on arbitrary scale.

TABLE X

Carbon–proton couplings in helenalin (*I*) and carbon–proton torsion angles calculated from X-ray data of helenalin (*I*) and plenolin (*II*)

Carbon	Direct coupling		Geminal couplings		Vicinal couplings		Torsion angles	
							I	*II*
C1	C1–H1	127	C1–C2–H2	[a]	C1–C2–C3–H3	[a]	–177	–179
			C1–C10–H10	[a]	C1–C5–C6–H6	[a]	176	–176
					C1–C10–C9–H9α	[a]	156	54
					C1–C10–C9–H9β	[a]	–86	170
					C1–C10–C14–H14	[a]	[e]	[e]
					C1–C5–C15–H15	[a]	[e]	[e]
C2	C2–H2	165.2	C2–C1–H1	9.1	C2–C1–C10–H10	~1	54	79
			C2–C3–H3	3.2				
C3	C3–H3	173.8	C3–C2–H2	3.2	C3–C2–C1–H1	4.5	–106	94
C4	–	–	C4–C3–H3	~5	C4–C5–C1–H1	~0	–103	–89
					C4–C3–C2–H2	13	–177	–179
					C4–C5–C6–H6	<1	–70	–60
					C4–C5–C15–H15	~1	[e]	[e]
C5	–	–	C5–C1–H1	[b]	C5–C1–C2–H2	[b]	166	169
			C5–C6–H6	[b]	C5–C4–C3–H3	[b]	–171	–163
			C5–C15–H15	[b]	C5–C1–C10–H10	[b]	–70	–42
					C5–C6–C7–H7	[b]	–147	–138
C6	C6–H6	144.4	C6–C7–H7	[c]	C6–C5–C1–H1	[c]	14	33
					C6–C7–C8–H8	[c]	–64	–149
					C6–C5–C15–H15	[c]	[e]	[e]
C7	C7–H7	131	C7–C6–H6	~0	C7–C8–C9–H9α	~2	160	–59
			C7–C8–H8	~0	C7–C8–C9–H9β	~4	42	–177
					C7–C11–C13–H13	~7	175	[f]
					C7–C11–C13–H13′	~2	–7	[f]
C8	C8–H8	151.4	C8–C7–H7	4.4	C8–C7–C6–H6	~7.2	–151	–141
			C8–C9–H9α	~7.2	C8–C9–C10–H10	~3	153	50
			C8–C9–H9β	~7.2				
C9	C9–H9α	126.6	C9–C8–H8	[d]	C9–C10–C1–H1	[d]	–70	–44
	C9–H9β	126.6	C9–C10–H10	[d]	C9–C8–C7–H7	[d]	156	88
					C9–C10–C14–H14	[d]	[e]	[e]

TABLE X
(*Continued*)

Carbon	Direct coupling		Geminal couplings		Vicinal couplings		Torsion angles	
							I	*II*
C10	C10–H10	129.4	C10–C9–H9α	~4.2	C10–C1–C2–H2	~1.5	36	41
			C10–C9–H9β	~4.2	C10–C9–C8–H8	~4.2	40	180
			C10–C1–H1	~5				
			C10–C14–H14	~1				
C11	–	–	C11–C7–H7	9.1	C11–C7–C6–H6	~3.0	–23	–20
			C11–C13–H13	~2.7	C11–C7–C8–H8	~2.0	162	82
			C11–C13–H13′	1.1				
C12	–	–	–	–	C12–C11–C7–H7	~2.8	92	144
					C12–O8–C8–H8	~2.8	–149	–89
					C12–C11–C13–H13	6.8	5	[f]
					C12–C11–C13–H13′	13.3	179	[f]
C13	C13–H13	160.6	–	–	C13–C11–C7–H7	4.9	–83	[f]
	C13–H13′	163.9						
C14	C14–H14	126.1	C14–C10–H10	4.3	C14–C5–C1–H1	2.7	53	136
					C14–C10–C9–H9α	~4.3	33	–67
					C14–C10–C9–H9β	~4.3	151	49
C15	C15–H15	129.9	–	–	C15–C10–C1–H1	3.2	144	–142
					C15–C5–C6–H6	1.1	45	55

The sum (Σ) of geminal and vicinal coupling constants could be determined only: [a] Σ ≈ 31 Hz; [b] Σ ≈ 24.5 Hz; [c] Σ ≈ 21 Hz; [d] Σ ≈ 24 Hz. [e] Three angle values for methyl protons H-14 and H-15 in crystal are not indicated – a fast rotation of methyl group occurs in solution. [f] In *II* there are methyl protons instead of exomethylene ones in position 13.

(acetone), respectively. While in the first case (in methanol) we have observed the expected lowfield shifts at C(4) and C(12) (when referenced to $CDCl_3$) in the second case (in acetone) the expected upfiled shift of C(6)–OH was absent (see Table XI).

EXPERIMENTAL

X-Ray Analysis

Helenalin (*I*), $C_{15}H_{18}O_4$, M_r = 262.305, $F(000)$ = 280, room temperature; monoclinic form (*Ia*), $P2_1$, a = 6.130(1), b = 8.206(1), c = 13.830(2) Å, β = 98.95(1)°, V = 687.2(2) Å^3, D_m = 1.26, D_x = 1.27 g cm^{-3},

Z = 2, λ(CuKα) = 1.54178 Å, μ = 0.71 mm^{-1}, R = 0.041 (wR = 0.063) for 1 791 observed reflections; orthorhombic form (*Ib*), $P2_12_12_1$, a = 7.816(1), b = 7.847(1), c = 22.148(4) Å, V = 1 358.4(4) Å^3, D_m = 1.26, D_x = 1.28 g cm^{-3}, Z = 4, λ(CuKα) = 1.5418 Å, μ = 0.71 mm^{-1}, R = 0.046 (wR = 0.057) for 1 613 observed reflections.

X-Ray data for both polymorphs were collected on a Syntex $P2_1$ diffractiometer with graphite monochromated CuKα radiation. Accurate cell constants were determined from setting angles of 15 reflections; ω – 2θ scans and variable scan speed were applied. Two standard reflections remeasured every 100 reflections showed no change in intensity greater than 2σ(I). The background and integrated intensity for each reflection were evaluated from a profile analysis according to Lehmann and Larsen[19] using the PRARA program[20]. Lorentz and polarization factors were applied but no absorption correction. Structure solved by direct methods using SHELXS86 (ref.[21]); non-hydrogen atoms refined anisotropically. Positions of hydrogen atoms were calculated, H atoms were assigned a common isotropic thermal parameter U = 0.07 Å^2 and allowed to ride on parent carbon atoms with the exception of the hydroxyl and methylene hydrogens which were located from a difference Fourier map and were allowed to vary; methyl groups were set up and refined as a rigid group. In the final stages of the refinement an empirical isotropic extinction parameter x was introduced to correct the calculated structure factors by multiplying them by a factor $1 - xF_c^2/\sin\theta$. The solution and refinement of the two structures was based, at first, on the set of reflections in which Bijvoet pairs were aver-

TABLE XI

Carbon-13 chemical shifts of helenalin in solid state and the shielding differences connected with different types of the intermolecular hydrogen-bondings in solid state and in solution

Carbon	Solid state ^{13}C chemical shifts		^{13}C Chemical shifts differences[a]				
	Ia	*Ib*	*Ia* – *Ib*	*Ia* – *I*(Chl)	*Ib* – *I*(Chl)	*I*(Me) – *I*(Chl)	*I*(Ac) – *I*(Chl)
C-1	53.84	54.34	–0.50	2.50	3.00	1.70	1.00
C-2	169.04	164.96	4.08	5.16	1.08	2.00	–0.03
C-3	128.78	131.66	–2.88	–1.19	1.67	0.30	0.04
C-4	217.82	211.52	6.30	5.61	–0.69	1.59	–1.44
C-5	55.60	57.58	–1.98	–2.38	–0.40	0.50	–0.29
C-6	68.26	67.62	0.64	–5.84	–6.48	1.34	0.92
C-7	52.18	48.65	3.53	1.42	–2.11	1.98	1.37
C-8	78.59	79.40	–0.81	0.44	1.25	2.40	0.97
C-9	36.98	37.75	–0.77	–2.42	–1.65	1.67	1.36
C-10	26.50	27.80	–1.30	0.33	1.63	1.28	0.85
C-11	136.84	137.32	–0.48	–1.02	–0.54	2.56	2.38
C-12	169.75	172.11	–2.36	0.01	2.37	2.44	0.46
C-13	120.99	122.18	–1.19	–2.06	–0.87	0.29	–0.98
C-14	19.24	21.24	–2.00	–0.91	1.09	0.31	0.25
C-15	18.62	18.14	0.48	–0.07	–0.55	0.58	0.25

[a] Chl chloroform, Me methanol, Ac acetone.

aged, with no corrections for anomalous dispersion effects. The parameters obtained were used in two structure factor calculations, without and with inverted signs of corrections[22]. The *wR* values obtained for the two models differed each time by only 0.0002 but the configuration with lower *wR* value was always in line with the absolute configuration assigned to helenalin on the basis of chemical correlation[2]. After enantiomorph definition the refinement was carried out on the full data sets (Bijvoet pairs not averaged) with oxygen and carbon atoms allowed for dispersion. Refinement using *F* magnitudes by full-matrix least squares of SHELX76 (ref.[23]). Atomic scattering factors for C, H and O those stored in SHELX76. Program PARST (ref.[24]) was used for geometry calculations, as implemented in CRYSRULER package[25].

Single crystals *Ia* were grown by slow evaporation from chloroform–ether solution; crystal ca $0.2 \times 0.3 \times 0.5$ mm was selected for data collection. 2 084 reflections measured, $2\theta < 115°$, 1 791 observed [$|F_o| > 3.92\sigma(|F_o|)$], index range *h* 0/6, *k* –8/8, *l* –15/15. Empirical extinction parameter *x* refined to a value 7.3(1) . 10^{-6}. Parameter $R = 0.041$; $wR = 0.063$ for 1 791 reflections and 190 parameters [$w^{-1} = \sigma^2(F) + 0.0001F^2$], $\Delta/\sigma = 0.01$; residual electron density in difference map within –0.24 and +0.29 e Å^{-3}.

Single crystals *Ib* were grown by slow evaporation from methanol–ether solution containing in addition to helenalin a small amount of other sesquiterpene lactones as impurities; crystal ca $0.15 \times 0.25 \times 0.30$ mm was selected for data collection. 2 102 reflections measured, $2\theta < 115°$, 1 613 observed [$|F_o| > 3.92\sigma(|F_o|)$], index range *h* 0/8, *k* 0/8, *l* –24/24. Empirical extinction parameter *x* refined to a value 1.7(1) . 10^{-6}. Parameter $R = 0.046$; $wR = 0.057$ for 1 613 reflections and 191 parameters [$w^{-1} = \sigma^2(F) + 0.0003F^2$], $\Delta/\sigma = 0.02$; residual electron density in difference map within –0.24 and +0.22 e Å^{-3}.

NMR Spectra

1D solution ^{1}H and ^{13}C NMR spectra of helenalin (*I*) were measured on a Varian UNITY-500 FT NMR spectrometer (^{1}H at 500 MHz; ^{13}C at 125.7 MHz frequency) in perdeuterated acetone, benzene, chloroform, cyclohexane, dichloromethane, methanol and pyridine with tetramethylsilane as internal reference. The most of additional NMR experiments – (i) proton 2D-ROESY, (ii) inversion-recovery measurement of ^{13}C relaxation times T_1, (iii) ^{13}C proton-coupled spectra and selective proton-decouplings, (iv) 2D ^{1}H-^{13}C correlated HMQC and HMBC – were done in $CDCl_3$ using a standard Varian pulse sequence software. Low temperature ^{1}H NMR spectra were run in CD_2Cl_2 at 0, –20, –40 and –60 °C.

Solid state ^{13}C cross-polarization/magic angle spinning NMR spectra of monoclinic and orthorhombic form of helenalin (*I*) were measured at room temperature with a Bruker MSL-200 spectrometer (at 50.3 MHz), in Al_2O_3 rotors at a spinning frequency 4.5 kHz. Sample amount was about 200 mg, the rotor space was filled up with Teflon filling. Number of scans was about 4 000, the contact time 3 ms, pulse repetition time 10 s, spectral width 20 kHz and number of points 16 k. Chemical shifts referred to the carbonyl band of glycine at $\delta = 176.0$ ppm by sample replacement.

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