^{1}H NMR Study of the Stereochemistry of Lubimin and Related Vetispirane Sesquiterpenoids

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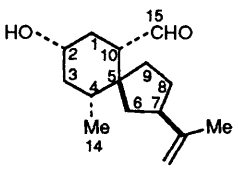
The stereochemistry at C-2 and C-10 in lubimin, 15-dihydrolubimin and 3-hydroxylubimin derivatives has been investigated by ^{1}H NMR spectroscopy. Using parent compounds and acetyl derivatives (24 compounds) definitive spectroscopic assignments have been made. Nine oxidised derivatives (2- and 3-keto functionalisation) have also been investigated. The usefulness of diagnostic features in the spectra are illustrated and the conformational homogeneity of the series verified.

The vetispirane lubimin (**1**)† is representative of a group of related sesquiterpenoids which have been isolated from fungal infected potato tubers and other solanaceous plants.[1–4] Early



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work on this class of compound was concerned largely with their characterisation and with the exploration of their biosynthetic interrelationships but there is now increasing interest in these compounds, some of which act as phytoalexins (antibiotics) *in planta*. Thus, the solution conformation of lubimin and related vetispiranes is of considerable importance and we report here a detailed investigation of 34 compounds in this series using principally ^{1}H NMR data obtained at 270 MHz. The investigation has revealed several diagnostic features for this group of compounds which are useful in the analysis of small biological samples containing epimeric mixtures. Spectroscopic data obtained previously on some of the compounds discussed in this work were derived from low-field spectra and are of limited value for conformational analysis.

Compounds (**3**)–(**5**) and (**7**)–(**34**) were synthesized from lubimin (**1**) or from 3-hydroxylubimin (**6**) obtained from natural sources.[5]

Results and Discussion

Lubimin is normally shown as structure (**1**) in biological reports. However, it is more useful for the present investigation to use formula (**2**) with the cyclohexane ring displayed in a normal chair conformation. The numbering employed by biochemists is non-systematic but will be retained in this work to avoid confusion and is shown in (**1**).

There are five chiral centres in (**2**) and six in compounds with $R^5 \neq H$. However, the configurations at carbon atoms C-4, C-5, and C-7 are invariant throughout the whole series of compounds and, where C-3 is asymmetric, the configuration at that site is also invariant. Unfortunately the presence of a hydroxy substituent at C-3 changes the configurational label at C-2, C-4, and in certain cases C-5, and care must be exercised in interpreting the significance of these labels. Since this work is concerned mostly with configurational variation at C-2 and C-10 the labels for these centres are given in Table 1. These configurational complexities may be summarised by indicating that (**2**) is (2*S*,4*R*,5*S*,7*R*,10*S*)-lubimin, (**6**) is (2*R*,3*R*,4*S*,5*S*,7*R*,10*S*)-3-hydroxylubimin, and (**20**) is (2*R*,3*R*,4*S*,5*R*,7*R*,10*S*)-3-hydroxy-15-dihydrolubimin although these three compounds have the same configurations at all centres.

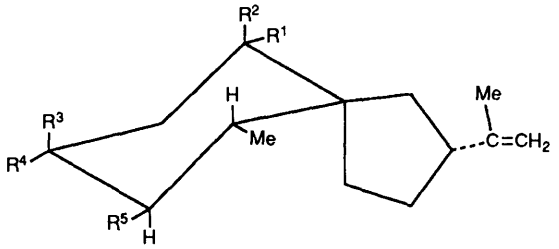
Even at 270 MHz the ^{1}H NMR spectra of these sesquiterpenoids are very complex and the range 1.0–2.0 ppm covers chemical shifts of up to ten protons. The shift data of diagnostic value are given in Table 1 for those cases where assignments were possible. (In several cases the minor isomers were examined only in admixture with the major isomer.) The useful coupling constants are given in Table 2. Many additional values could be obtained for particular protons and these are noted in the text.

The chemical shifts for the isopropenyl group showed very little variation; the methyl group occurred as a broadened singlet in the range 1.70–1.76 ppm and the vinyl protons as a multiplet in the range 4.70–4.75 ppm and these values are not tabulated.

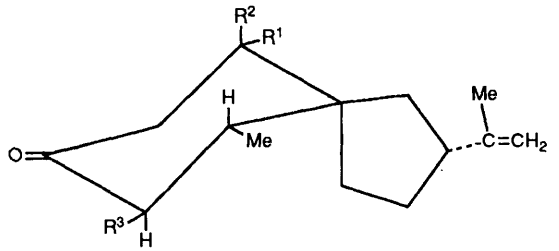
Coupling constants have been calculated for several structures using the Altona[6] equation which is a modification of the established Karplus relation to take account of the electronegativity of substituents. The labels a and pa are used to indicate axial and pseudoaxial positions and e and pe for the corresponding equatorial positions.

Lubimin (**2**) is a substituted spiro[4,5]decane and the conformation of the two rings can be discussed separately. The six-membered ring has three mutually *cis* substituents and thus the only contributing conformation is that shown in (**2**) in which the ring itself has C_{2v} symmetry and the substituents occupy equatorial positions. This symmetry is confirmed by the couplings to H-3 and by the relatively large ^4J value between H-1e and H-3e. Calculated values of $^3J_{1e,2}$ and $^3J_{1a,2}$ are 4.75 and 11.2 Hz, respectively, for an endocyclic torsion angle of 56° suggesting that this ring has the slight flattening typical of cyclohexane derivatives. The cyclopentane ring is more difficult to investigate since most of the protons in this ring have shifts in the range 1.5–1.7 ppm and little coupling data is accessible. Proton H-7 has couplings of *ca.* 6, 9, 9, and 11.5 Hz indicating an

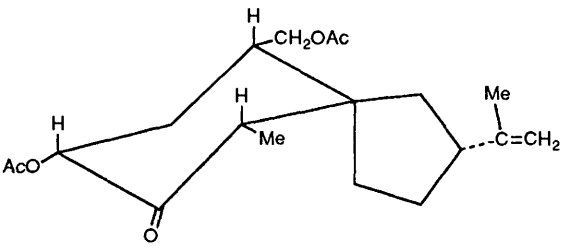
† The systematic name for lubimin is (2*R*,5*S*,6*S*,8*S*,10*R*)-8-hydroxy-10-methyl-2-prop-2-enylspiro[4,5]decane-6-carbaldehyde.



	R^1	R^2	R^3	R^4	R^5
(2)	CHO	H	H	OH	H
(3)	H	CHO	H	OH	H
(4)	CHO	H	H	OAc	H
(5)	H	CHO	H	OAc	H
(6)	CHO	H	H	OH	OH
(7)	H	CHO	H	OH	OH
(8)	CHO	H	H	OAc	OAc
(9)	H	CHO	H	OAc	OAc
(10)	CH_2OH	H	H	OH	H
(11)	H	CH_2OH	H	OH	H
(12)	CH_2OAc	H	H	OH	H
(13)	H	CH_2OAc	H	OH	H
(14)	CH_2OH	H	H	OAc	H
(15)	H	CH_2OH	H	OAc	H
(16)	CH_2OAc	H	H	OAc	H
(17)	H	CH_2OAc	H	OAc	H
(18)	CH_2OH	H	OH	H	H
(19)	H	CH_2OH	OH	H	H
(20)	CH_2OH	H	H	OH	OH
(21)	H	CH_2OH	H	OH	OH
(22)	CH_2OH	H	H	OAc	OAc
(23)	CH_2OAc	H	H	OAc	OH
(24)	CH_2OAc	H	H	OH	OAc
(25)	CH_2OAc	H	H	OAc	OAc



	R^1	R^2	R^3
(26)	CH_2OH	H	H
(27)	H	CH_2OH	H
(28)	CH_2OAc	H	H
(29)	H	CH_2OAc	H
(30)	CHO	H	H
(31)	H	CHO	H
(32)	CH_2OAc	H	OAc
(33)	H	CH_2OAc	OAc



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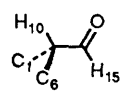
unsymmetrical environment for this site. This is in keeping with an envelope conformation with C-5 *trans* to the C-7 substituent *i.e.* the spiro-carbon is out of plane.

Inversion of the configuration at C-10 in lubimin to give 10-*epi*-lubimin (**3**) results in several significant changes to the proton spectrum. Repulsive 1,3-interactions between the axial formyl group at C-10 and H-2a and H-4a produces further general flattening of the cyclohexane ring (both *gauche* couplings to H-2 are increased by 0.2–0.3 Hz). Further distortion at C-10 is indicated by the *gauche* couplings of 2.4 and 5.4 Hz. These are both slightly larger than the values calculated for an endocyclic angle of 70° (1.8 and 4.3 Hz, respectively). In (**2**) the formyl group is equatorial and has the conformation shown in (**35**) with H-15 nearly antiperiplanar to H-10 giving rise to a large coupling, $^3J_{10,15}$ 3.2 Hz. In contrast, in the 10-*epi* isomer (**3**) the torsion angle between H-10 and H-15 is close to 90°, as shown in (**36**). The ^3J coupling constant is reduced to 0.95 Hz and four-bond coupling is developed between H-15 and H-1a (0.95 Hz). Conformation (**36**) minimises 1,2-interactions between the 10-CHO group and the five-membered ring. It also accounts for the significant deshielding of the pseudoequatorial proton H-6 which appears at unusually low field, δ 2.031, with couplings of 13.2, 6.9, and 1.8 Hz. This last coupling, $^4J_{6pe,8pe}$, indicates that these protons are nearly coplanar with C-7 and that the ring has an envelope conformation with the C-7 substituent out-of-plane. This is confirmed by the symmetry of the couplings to H-7 (*ca.* 6, 6, 12, and 12 Hz).



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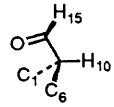

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The acetyl derivatives of (**2**) and (**3**) were examined as a mixture of isomers (**4**) and (**5**) and only a few assignments were possible for the minor isomer. The epimeric shift at the formyl proton is smaller (0.03 ppm) than in the non-acetylated species but a measurable shift was developed for the 14-Me group providing a convenient estimate of the isomer ratio (in this case 21 : 4, see the Experimental section). There appears to be no significant conformational consequence of acetylation of the 2-OH group.

Formation of the 3-hydroxy derivative of lubimin occurs stereospecifically *in vivo* to place the hydroxy group *trans* to the substituents at C-2 and C-4. Thus the conformation of the six-membered ring in 3-hydroxylubimin (**6**) is the same as in the parent system, with all substituents occupying equatorial sites. This is established by the coupling data for (**6**) and the 10*R* (*epi*) form (**7**). A 1,2-repulsive interaction between the vicinal hydroxy groups probably results in a slight increase in the flattening of the ring since the observed value of $J_{2a,3a}$ is close to the calculated value of 8.8 Hz for an endocyclic torsion angle φ[C-1,C-4] of 54°. (The major factor accounting for the 2.2 Hz reduction in $J_{2a,3a}$ relative to lubimin is the presence of an extra OH substituent).

The normal isomer of 3-hydroxylubimin (**6**) has a similar conformation for the formyl group as was observed in (**2**), $^3J_{10,15} = 2.9$ Hz. The presence of the 3-hydroxy group deshields H-1a, H-1e, and H-10 and shields H-2a as expected, and similar shifts are observed in the *epi*-isomer (**7**) for H-1a, H-1e, and H-2a but H-10e is unexpectedly shielded relative to (**3**). Long range coupling of 0.9 Hz between the formyl proton and H-1a in (**7**) indicates a conformation of type (**36**) and this is confirmed by the appearance of H-6pe at δ 2.092 with couplings of 13.2, 7.0, and 1.8 Hz. Thus the isomers (at C-10) of 3-hydroxylubimin show the same conformational preferences as do the corresponding isomers of lubimin and can thus be expected to demonstrate similar *in vivo* selectivity.

Table 1. ^{1}H Chemical shifts[a] in lubimin and related compounds.

Compound	Configuration[b]	H-1e	H-1a	H-2a	H-3e	H-3a	H-7	H-8pa	H-10[c]	H-15	H-15′	Me-14	Ac
(2)	2*S*,10*S*	1.972	1.539	3.700	1.7	1.110	2.43	1.323	2.236a	9.807		0.950	
(3)	2*S*,10*R*	2.237	1.7	3.738	1.7	1.157	2.43	1.415	2.445a	9.860		0.951	2.031[d]
(4)	2*S*,10*S*	2.01	1.7	4.759	1.7	1.163	2.43		2.340a	9.841		0.947	2.031
(5)	2*S*,10*R*			4.80			2.43			9.873		0.952	2.017
(6)	2*R*,10*S*	2.007	1.7	3.470		3.038	2.45	1.32	2.346a	9.808		1.073	
(7)	2*R*,10*R*	2.331	1.75	3.462		3.085	2.45		2.39e	9.871		1.078	2.092[d]
(8)	2*R*,10*S*	2.12		4.83		4.75		1.32	2.43a	9.835		0.931	2.017, 2.057
(9)	2*R*,10*R*									9.885		0.92	
(10)	2*S*,10*S*	2.247	1.147	3.655	1.7	1.053	2.37			3.360	3.965	0.927	
(11)	2*S*,10*R*	2.155		3.882						3.63	3.74	0.882	
(12)	2*S*,10*S*	2.05	1.165	3.64		1.050	2.38			3.82	4.35	0.93	2.05
(13)	2*S*,10*R*	2.05		3.87			2.38			4.11	4.13	0.89	2.07
(14)	2*S*,10*S*	2.295	1.250	4.793		1.115	2.43			3.360	3.85	0.92[e]	2.02
(16)	2*S*,10*S*	2.09	1.250	4.735		1.120	2.37			3.82	4.34	0.93[f]	2.02, 2.04
(18)	2*R*,10*S*	2.05		4.12[g]			2.36			3.34	3.98	0.882	
(19)	2*R*,10*R*			3.94[g]						3.61	3.75	0.942	
(20)	2*R*,10*S*	2.29		3.436		2.995	2.41			3.338	3.940	1.059	
(21)	2*R*,10*R*			3.65		3.093	2.39			3.69	3.79	1.015	
(22)	2*R*,10*S*	2.36		4.83		4.71	2.39			3.36	3.94	0.905	2.01, 2.05
(23)	2*R*,10*S*	2.175		4.638		3.175	2.43			3.785	4.32	1.08	2.05, 2.09
(24)	2*R*,10*S*	2.203		3.549		4.477	2.40			3.788	4.351	0.915	2.05, 2.13
(25)	2*R*,10*S*	2.200		4.81		4.71	2.41			3.775	4.35	0.913	2.01, 2.05, 2.05
(26)	10*S*	2.657	2.256		2.15	2.11	2.44			3.610	3.978	0.999	
(27)	10*R*									3.582	3.810	0.937	
(28)	10*S*	2.53	2.25		2.1	2.1	2.44			3.88	4.39	1.01	2.05
(29)	10*R*	2.52	2.1		2.3	2.1				3.87	4.26	0.94	2.05
(30)	10*S*	2.72	2.66		2.22	2.16	2.5	1.395	2.36a	9.92		1.014	
(31)	10*R*								2.94e	9.86		0.998	
(32)	10*S*	2.665	2.400			4.867	2.48			3.862	4.410	1.072	2.18, 2.19
(33)	10*R*					4.89						1.020	
(34)	2*R*,10*S*	2.48		5.219					2.39a	3.84	4.34	1.07	2.08, 2.16, 2.63[h]

[a] All data are determined for solutions in $CDCl_3$, with $SiMe_4$ as the reference, at 270.168 MHz. [b] Configurational labels as discussed in the text. [c] Values labelled to indicate axial (a) or equatorial (e). [d] Value of δ(H-6pe). [e] In *epi*-isomer (**15**) δ(Me-14) = 0.88. [f] In *epi*-isomer (**17**) δ(Me-14) = 0.90. [g] Value of δ(H-2e). [h] Value of δ(H-4).

Table 2. Selected coupling constants *J*/Hz in lubimin and related compounds.[a]

Compound	$J_{1e,1a}$	$J_{1e,2}$	$J_{1a,2}$	$J_{1e,3e}$	$J_{1e,10}$	$J_{1a,10}$	$J_{2,3e}$	$J_{2,3a}$	$J_{3a,4a}$	$J_{10,15}$	$J_{10,15'}$
(2)	12.9	4.7	11.0	2.4	3.5	12.9	4.6	11.0		3.2	
(3)	13.2	4.9	11.0	2.1	2.4	5.4	4.9	11.0		0.95	
(6)	13.2	5.0	11.5		3.6	13.0		8.8	10.8	2.9	
(7)	13.3	5.1	11.9		2.3	5.5		8.9	10.6	0.9	
(10)	12.5	4.6	11.1	2.4	3.2		4.6	11.1		8.9	3.4
(11)		4.6	11.1				4.6	11.1		9.5	3.5
(18)		3.0	3.0				3.0	3.0		9.0	3.5
(19)		4.3	8.5				4.3	8.5		8.9	4.0
(20)	12.9	5.1	11.5		3.2			8.8	10.7	9.0	3.4
(21)		5.2	11.5					8.8	10.8	9.0	3.5
(22)	12.9	5.2	11.5		3.4			9.2	10.9	9.2	3.5
(23)	12.9	5.2	11.4		3.4			9.2	11.2	9.2	3.5
(26)	14.8			1.8	4.2	12.6				8.3	3.7
(30)	12.5			0.5	2.1	12.9				1.95	
(31)					5.2	8.4				1.2	
(32)	14.2			1.2[b]	4.1	13.4			12.8	9.1	3.6
(34)	13.0	7.2	12.7		3.6	12.5		0.9[c]		9.0	3.6

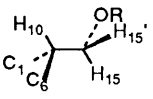
[a] Protons H-2 and H-10 can be axial or equatorial as indicated in Table 1. [b] $J_{1a,3a}$. [c] $J_{2a,4a}$.

Acetylation of a mixture of (**6**) and (**7**) in the ratio 24:1 gave diacetates (**8**) and (**9**) but only limited data were accessible for the minor isomer. In both isomers the formyl proton is slightly deshielded by the acetyl groups but the couplings are unaffected indicating no change in conformation.

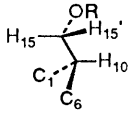
Reduction of a mixture of lubimin (**2**) and 10-*epi*-lubimin (**3**) (3:2) regiospecifically at the formyl group gave the dihydro derivatives 15-dihydrolubimin (**10**) (DHL) and 10-*epi*-15-dihydrolubimin (**11**) (3:2). The abundant isomer (**10**) has the same 2*S*,10*S* configuration as its precursor lubimin and the conformation of the cyclohexane ring is also unchanged, as indicated by the couplings in the C-1, C-2, C-3 fragment. Fewer individual protons can be discerned in the spectra of (**10**) and the *epi*-isomer (**11**) but the 14-Me group shows a diagnostic upfield shift of *ca* 0.05 ppm between the 10*S* and 10*R* forms. This difference is maintained in the 2- and 15-*O*-acetyl derivatives

(**12**)–(**17**) and provides the most convenient measure of the 10*S*:10*R* ratio in these compounds. The acetyl derivatives 2-*O*-acetyl-DHL (**14**) and 2,15-di-*O*-acetyl-DHL (**16**) only contained a small amount of the 10-*epi*-isomers (**15**) and (**17**), respectively, identified by the Me-14 doublet. The other data for the acetyl derivatives confirm that *O*-substitution at either hydroxy group in DHL has no effect on conformation. Thus, the DHL species is likely to have the same ring and side chain conformation whether it exists *in vivo* as a glycoside or the free diol.

It is evident from the coupling data that the CH_2OH in (**10**) adopts the conformation shown in (**37**), with the hydroxy group synclinal to the C-1, C-10 bond. This conformation minimises the interactions of the CH_2OH group with the cyclopentane ring and, in the absence of rotational averaging, accounts for the substantial deshielding of H-15′ relative to H-15, these protons being, respectively, quasiequatorial and quasiaxial with respect to the cyclohexane ring. In contrast, protons H-15 and H-15′ have very similar chemical shifts in 10-*epi*-DHL (**11**). This is consistent with the conformation shown in (**38**) which is indicated also by the coupling data. Again the hydroxy group is synclinal to the C-1, C-10 bond. A stereospecific coupling $^4J_{1a,H-15'}$ 1.0 Hz is observed, also indicative of a near-planar H–C–C–C–H pathway.



(**37**)



(**38**)

DHL (**10**) was also obtained by reduction of isolubimin epimers (**26**) and (**27**) to give a mixture of 2*R*,2*S* stereoisomers which were separated chromatographically. This afforded one fraction (A) which was a mixture of the usual 2*S*,10*S* and 2*S*,10*R* isomers (**10**) and (**11**) in the ratio 3:2 and a second fraction (B) which was a mixture of the 2*R*,10*S* and 2*R*,10*R* isomers, 2-*epi*-DHL (**18**) and 2-*epi*-10-*epi*-DHL (**19**), in the ratio 7:13 *i.e.* the 10-*epi* form was predominant. Examination of fraction (B) gave the data included in the tables. The minor species in (B), 2-*epi*-DHL (**18**) has a chair conformation for the cyclohexane ring with four equal *gauche* couplings to H-2, $^3J = 3.0$ Hz. Calculated values of $^3J_{1e-2e}$ and $^3J_{1a-2e}$ are 3.1 and 2.7 Hz, respectively, for an endocyclic torsion angle [C-10,C-3] of 53° and both couplings are 3.0 Hz for an angle of 52°. Evidently the ring is slightly flattened by the repulsive interactions arising from the axial 2-hydroxy group. The most significant difference in the chemical shifts of the ring protons between (**18**) and (**10**) is the 0.46 ppm downfield shift for H-2 corresponding to an axial to equatorial conversion. An essentially unaltered conformation for the CH_2OH substituent is confirmed by the similarity of the chemical shifts and the couplings for H-15 and H-15′ to the analogous parameters in (**10**).

The major component of the fraction (B) was 2-*epi*-10-*epi*-DHL (**19**). This compound cannot maintain a chair conformation since there is a severe 1,3 interaction between the axial 2-OH and 10-CH_2OH groups. Proton H-2 is a septet corresponding to two couplings of *ca.* 4.3 Hz and two of 8.5 Hz. Calculation of the values of the couplings to H-2 indicates that the observed data do not correspond to the boat form with the fold axis through C-2 nor to the closely related twist forms. It is notable that there is no four-bond coupling to H-15′ [*cf.* (**11**)] although H-15 is significantly broadened, indicative of long range coupling. Furthermore, the chemical shift of H-2 does not correspond to an equatorial environment. Thus, it is likely that several twist forms contribute to the conformation of (**19**). The CH_2OH group maintains conformation (**38**), the same as that found in the 2*S* isomer but the 14-Me substituent is downfield in (**19**) relative to (**18**) thus reversing the order observed in the 2*S* isomers.

Reduction of 3-hydroxylubimin (**6**) and 10-*epi*-3-hydroxylubimin (7) gave 3-hydroxy-15-dihydrolubimin (**20**) and 10-*epi*-3-hydroxy-15-dihydrolubimin (**21**). This reduction was achieved synthetically for the present work although (**20**) has been obtained by us from a biological source.[5] The data for (**20**) and (**21**) show that the conformation of both isomers is essentially the same as in the corresponding lubimin series. Furthermore, the Me-14 group exhibits the usual diagnostic shift difference for the DHL series and the 10-CH_2OH group has conformations (**37**) and (**38**) for the normal and *epi* isomers respectively. Notably H-3a is at considerably higher field than the notionally similar proton H-2a, reflecting the significant shielding effect of the cyclopentane ring.

Acetylation of (**6**) followed by reduction gave 2,3-di-*O*-acetyl-3-hydroxylubimin (**22**) whilst partial acetylation of (**20**) gave the 2,15-di-*O*-acetyl, 3,15-di-*O*-acetyl, and 2,3,15-tri-*O*-acetyl derivatives (**23**)–(**25**), respectively. The 14-Me group is shielded by the 3-acetoxy group but little affected by the other ester groups. Acetylation at O-3 results also in a greater deshielding of H-3 (by *ca.* 0.3 ppm) than is the case for acetylation at O-2 and this difference is apparent also in (**25**), and in the data for (**6**) and (**8**).

A reduced conformational mobility for the 3-*O*-acetyl group is implied by these results; the major contributing structure probably has the acetyl group synperiplanar to H-3 with the carbonyl group antiperiplanar to the C-3,O-3 bond. The restricted rotational volume at this site may have significant implications for enzymatic access to this site in lubimin.

As expected, compounds (**20**)–(**25**) show no evidence of coupling between H-1e and H-3a. However, in derivative (**24**) H-3a shows fine structure which may be due partly to coupling to H-1a. Second-order effects arise from near coincidence of chemical shifts.

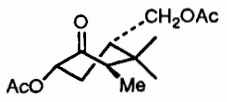
Biological oxidation of lubimin and its derivatives with formation of a cyclohexanone ring has been reported[7,8] and we include several such compounds in the present survey. Isolubimin (**26**) and its 10-*epi* isomer (**27**) were obtained as a mixture (3:2) by oxidation of a mixture of (**12**) and (**13**) (3:2) followed by deacetylation. These isomers were separated chromatographically but (**27**) was isolated only in very small amount. The usual upfield shift of the 14-Me group is apparent for the *epi*-isomer and a similar shift is observed in the acetyl derivatives (**28**) and (**29**). The CH_2OH group has the usual conformation, (**37**) in the normal isomer and (**38**) in the *epi*-isomer, as indicated by the coupling constants. The 2-keto group deshields H-15 in (**26**) (by 0.25 ppm) and H-15′ in (**27**) (by 0.1 ppm) confirming that the cyclohexanone ring is in the chair conformation. Calculated values of $^3J_{1a,10}$ and $^3J_{1e,10}$ are 12.1 and 4.0 Hz, respectively, for an endocyclic torsion angle of 56° indicating that the ring is not distorted significantly, relative to DHL. The decrease of $^4J_{1e,3e}$ to 1.8 Hz from 2.4 Hz in DHL is due to the electronegativity of the carbonyl group.

Oxidation of the mixture (3:2) of (**26**) and (**27**) with $Ru(Ph_3P)_3Cl_2$ gave 2-dehydrolubimin (**30**) and 10-*epi*-2-dehydrolubimin (**31**) in the same ratio (3:2). In another experiment (**26**) gave a mixture of (**30**) and (**31**) in the ratio, 5:1. The 14-Me groups are separated by less than the usual 0.05 ppm and the formyl protons have reversed shifts relative to lubimin isomers (**2**) and (**3**). This is mainly due to a downfield shift of H-15 in the normal isomer, (**30**). There is also a reduction in $J_{10,15}$ to 60% of the value in lubimin, suggesting a rotation of the formyl group by *ca.* 60° relative to conformation (**35**) placing the carbonyl bond antiperiplanar to the C-1,C-10 bond. Coupling to the formyl proton $J_{10,15}$ (1.2 Hz) in the 10-*epi*-isomer (**31**) is increased relative to (**3**) and no coupling is

observed with H-1a. Thus, this group adopts a conformation with the exocyclic carbonyl bond again antiperiplanar to the C-1,C-10 bond, a 120° rotation relative to conformation (**36**), to reduce the severe dipole repulsion between carbonyl groups. There is also significant distortion of the chair conformation as shown by couplings to H-10. No one conformation of the cyclohexane ring corresponds to these couplings and several twist forms must be involved.

Oxidation of 2,15-di-*O*-acetyl-3-hydroxy-15-dihydrolubimin (**23**) and 3,15-di-*O*-acetyl-3-hydroxy-15-dihydrolubimin (**24**) with Jones' reagent or pyridinium chlorochromate[9] gave 2,15-di-*O*-acetyl-3-oxo-15-dihydrolubimin (**34**) and 3,15-di-*O*-acetyl-2-oxo-3-hydroxy-15-dihydrolubimin (**32**), respectively. In the 2-oxo derivative (**32**) the CH_2OAc group maintains the normal conformation (**37**), but both H-15 and H-15′ are deshielded by the endocyclic carbonyl group. The presence of a 3-acetoxy group results in a significant distortion of the cyclohexanone ring due to a repulsive interaction with the 2-oxo and 4-methyl groups. This accounts for the increase in $J_{1a,10}$ [*cf.* (**26**)] and for the existence of a four-bond coupling (*ca.* 1 Hz) between H-3a and H-1a. The spectrum indicated the presence of *ca.* 3% of the 10-*epi*-isomer (**33**), which has the usual 0.15 ppm upfield shift of the Me-14 group.

In (**34**) the CH_2OAc group has the normal conformation (**37**) but the ring is forced into a twist form by the interaction of the ketone group with the substituents at C-2 and C-4. Although many forms could contribute, conformation (**39**) accounts for



(**39**)

the existence of *gauche* and *trans* couplings to H-2, for a significant long range coupling $^4J_{2a,4a}$ (0.9 Hz), for the substantial deshielding of these same protons, and for the lack of any effect of the keto group on the shift of the 14-Me group. Normal values of the couplings in the C-1,C-10 fragment confirm that pseudorotation in (**34**) is highly restricted, as seems to be characteristic of most of these derivatives of lubimin.

This survey of the spectral features of lubimin and derivatives has demonstrated the following. (*a*) In most species the six-membered ring is a slightly flattened chair. The exceptions are the cyclohexanone derivatives (**31**) and (**34**). (*b*) The 10*R* (*epi*) isomers can be distinguished from the 10*S* isomers by a diagnostic upfield shift of Me-14 in the latter case. (*c*) In dihydrolubimins (CHO reduced to CH_2OH) the CH_2OH group has a fixed orientation dependent only on the configuration at C-10. (*d*) Hydroxylation at C-3 has no effect on any of (*a*), (*b*), or (*c*). (*e*) *O*-Substitution at O-2, O-3, or O-15 has no significant effect on conformation.

However, the O-3 site is less accessible. These results imply that the lubimin system is conformationally well behaved and that the biochemistry of this class of sesquiterpenoid is unlikely to be species dependent.

Experimental

NMR spectra were recorded at 270.168 MHz on a JEOL GX 270 NMR spectrometer using 0.01–0.05 mol dm^{-3} solutions in $CDCl_3$. TLC was carried out on 0.5 mm Rhodamine 6G impregnated silica gel G plates developed in either solvent A (EtOAc–cyclohexane 1:1) or B (EtOAc–propan-2-ol 9:1). Compounds on a developed plate were located by examination of the plate under UV light of wavelength 254 nm and recovered from the gel with Et_2O. All solvents were AnalaR grade and were redistilled before use. For NMR data for all compounds see Tables 1 and 2.

Lubimin (**2**), 10-epi-*Lubimin* (**3**), 3-*Hydroxylubimin* (**6**), *and* 10-epi-3-*Hydroxylubimin* (**7**).—Potato tubers var. Kennebec (6 kg) were washed, surface sterilized with 10% Everchlor for 30 min, rinsed with water and EtOH and allowed to dry. The tubers were sliced into 5 mm thick sections which were washed in distilled water to remove excess starch. The sections (fresh wt. 5 kg) were blotted with tissues, placed in individual Petri dishes lined with moistened filter paper and kept in the dark for 24 h. They were then immersed in a 0.5 mmol dm^{-3} solution of the biotic elicitor sodium arachidonate[10] (made up from the free acid with equimolar $NaHCO_3$), returned to the Petri dishes and replaced in the dark at 17 °C for 4 days. After this time the sections were harvested and steeped in $CHCl_3$–MeOH (2:1) for 24 h. The resulting liquor was removed and a second extract obtained in the same way. The combined liquors were evaporated under reduced pressure to leave an aqueous extract which, after extensive extraction with Et_2O and reduction of the solvent under reduced pressure, afforded a crude oily residue. Preparative TLC of this material (solvent B) gave a crude oily mixture of (**2**) and (**3**) (51 mg), R_f 0.68, and crude rishitin (32 mg), R_f 0.55. Further purification of the mixture containing (**2**) and (**3**) was attempted by repeated TLC (solvent A, triple development). However, a small amount of a yellow plant pigment could not be resolved from the two compounds. NMR analysis showed that the ratio of (**2**) and (**3**) from this source was 21:4. Pure (**2**) and (**6**) were obtained from fruits of *Datura stramonium* as described elswhere.[5] Treatment of (**2**) with base (1 mol dm^{-3} KOH in EtOH for 2 h) and TLC (solvent A) gave a mixture of (**2**) and (**3**) (3:2), R_f 0.30 (see also below). Treatment of (**6**) (7 mg) with base and TLC (solvent B) gave (**6**) (3.7 mg), R_f 0.50 and (**7**) (2.5 mg), R_f 0.55. The MS and IR data for these compounds were identical with those reported previously.[1,3,11]

2-O-*Acetyllubimin* (**4**) *and* 10-epi-2-*O*-*Acetyllubimin* (**5**).—Treatment of a crude mixture of (**2**) and (**3**) (21:4) with Ac_2O and pyridine followed by TLC (solvent A) gave (**4**) and (**5**) (21:4), R_f 0.65; MS and IR data as reported.[1]

2,3-*Di*-O-*acetyl*-3-*hydroxylubimin* (**8**) *and* 10-epi-2,3-*Di*-O-*acetyl*-3-*hydroxylubimin* (**9**).—Acetylation (Ac_2O/py) of a mixture of (**6**) and (**7**) (24 : 1) followed by TLC (solvent A) gave a mixture of (**8**) and (**9**) (24 : 1), R_f 0.6; MS and IR as reported for (**8**).[1]

15-*Dihydrolubimin* (**10**) *and* 10-epi-15-*Dihydrolubimin* (**11**).—Reduction of a mixture of (**2**) and (**3**) (3:2) with $NaBH_4$ in EtOH followed by TLC (solvent A) gave (**10**) and (**11**), (3:2), R_f 0.06; MS and IR as reported.[1]

Isolubimin (**26**) *and* 10-epi-*Isolubimin* (**27**).—Treatment of a mixture of (**10**) and (**11**) (42 mg) with 80 mm^3 Ac_2O and 100 mm^3 pyridine in 2 cm^3 benzene for 4 h at room temperature, followed by TLC (solvent A), afforded: unchanged (**10**) and (**11**) (5.6 mg), R_f 0.06; 15-*O*-*acetyl*-15-*dihydrolubimin* (**12**) and 10-*epi*-15-*O*-acetyl-15-dihydrolubimin (**13**) (oil, 28.2 mg), R_f 0.33, broad band, ν_{max}(film) 3 410, 3 090, 2 950, 2 880, 1 790, 1 725, 1 648, 1 370, 1 240, 1 038, and 889 cm^{-1}; *m/z* 280 (M^+), 262, 238, 220 ($M - C_2H_4O_2$)$^+$, 202; 2-*O*-acetyl-15-dihydrolubimin (**14**) and 10-*epi*-2-*O*-acetyl-15-dihydrolubimin (**15**) (oil, 1.2 mg), R_f 0.44, ν_{max}(film) 3 450, 2 950, 2 880, 1 735, 1 370, 1 248, 1 032, and 890 cm^{-1}; *m/z* 280 (M^+), 220 ($M - C_2H_4O_2$)$^+$, 202; and 15-di-*O*-acetyl-15-dihydrolubimin (**16**) and 10-*epi*-2,15-di-*O*-acetyl-15-dihydrolubimin (**17**) (oil, 4.2 mg), R_f 0.62, ν_{max} 1 738, 1 715, 1 250, and 890 cm^{-1}, *m/z* 322 (M^+), 262 ($M - C_2H_4O_2$)$^+$, 220, 202 ($M - 2C_2H_4O_2$)$^+$. Oxidation of the

mixture of (**12**) and (**13**) (27 mg) with Jones' reagent and TLC (solvent A) gave 15-*O*-acetylisolubimin (**28**) and 10-*epi*-15-*O*-acetylisolubimin (**29**) (oil, 15.8 mg), R_f 0.65; ν_{max}(film) 3 085, 2 930, 2 880, 1 740, 1 720, 1 648, 1 370, 1 235, 1 038, and 890 cm^{-1}; *m/z* 278 (M^+), 236, 218 ($M - C_2H_4O_2$)$^+$, 203, 189. Deacetylation of this mixture with base (0.5 cm^3, 1 mol dm^{-3} KOH in EtOH plus one drop of pyridine for 2 h) followed by TLC (solvent A) gave *isolubimin* (**26**) and 10-*epi*-isolubimin (**27**) (oil, 13.8 mg, 32% overall yield), R_f 0.44; ν_{max}(film) 3 440, 3 082, 1 705, 1 645, and 889 cm^{-1}; *m/z* 236 (M^+), 218, 203. All of the above epimeric pairs were isolated as 3:2 mixtures (by NMR spectroscopy, see Table 1).

2-epi-15-*Dihydrolubimin* (**18**) *and* 2-epi-10-epi-15-*Dihydrolubimin* (**19**).—Reduction of a mixture of (**26**) and (**27**) (3:2, 5 mg) with $NaBH_4$ and careful separation by TLC (solvent A) afforded (**10**) and (**11**) (4.1 mg, 3:2), R_f 0.06, and (**18**) and (**19**) (0.7 mg, 7:13), R_f 0.09. The ratio of the C-2 epimers [*i.e.* (**10**) + (**11**):(**18**) + (**19**)] formed in the reaction was *ca.* 9:1 and not 3:7 as reported previously;[2] *m/z* (**10**) + (**11**) no 238 (M^+), 220, 202; (**18**) + (**19**) as reported.[4]

Lubimin (**2**) *and* 10-epi-*Lubimin* (**3**) *from* 15-*Dihydrolubimin*.—Treatment of a mixture of (**10**) and (**11**) (3:2) (10 mg) with $Ru(Ph_3P)_3Cl_2$ (1.6 mol equiv.) in benzene (0.5 cm^3) for 4 h effected the selective oxidation[12] of the C-15 primary alcohol. TLC of the mixture (solvent A) gave a mixture of (**2**) and (**3**) (3:2) which, after further TLC in the same solvent, gave pure (**2**) (3.9 mg), R_f 0.30, and pure (**3**) (2.7 mg), R_f 0.33. Treatment of pure (**10**) [obtained by $NaBH_4$ reduction of pure (**2**) with the organometallic reagent] resulted in a mixture of (**2**) and (**3**) (3:2 at C-10). This epimerization probably arose by keto–enol tautomerism of the –CHO group by a reaction mechanism similar to that observed when –CHO groups are treated with base. It was partly for this reason that much of the work reported herein was performed on epimeric mixtures.

2-*Dehydrolubimin* (**30**) *and* 10-epi-2-*Dehydrolubimin* (**31**).—Treatment of a mixture of (**26**) and (**27**) (7 mg, 3:2) with $Ru(Ph_3P)_3Cl_2$ as above, followed by TLC (solvent A), gave a mixture of (**30**) and (**31**) (4.6 mg, 3:2), R_f 0.59; ν_{max}(film) 3 085, 2 740, 1 720, 1 648, and 890 cm^{-1}; *m/z* 234 (M^+, 11%), 219(2), 205(2), 191(23), 163(19), 149(31), 135(42), 121(28), 107(47), 93(61), 79(49), 67(49), 55(47), 41(100).

3-*Hydroxy*-15-*dihydrolubimin* (**20**) *and* 10-epi-3-*Hydroxy*-15-*dihydrolubimin* (**21**).—Separate reductions of (**6**) and (**7**) with $NaBH_4$ at room temperature for 2 h (each 2 mg) and TLC (solvent B) afforded (**20**) and (**21**) respectively (both 1.8 mg), R_f 0.21; *m/z* 254 (M^+), 236, 218, 203. The sample of (**20**) prepared here by chemical means from (**6**) was identical in all respects with a sample of the compound recently isolated from a biological system for the first time.[5]

2,3-*Di*-O-*acetyl*-3-*hydroxy*-15-*dihydrolubimin* (**22**).—Reduction of (**8**) with $NaBH_4$ and TLC (solvent A) gave (**22**), R_f 0.30; MS and IR as reported.[1]

2,15-*Di*-O-*acetyl*-3-*hydroxy*-15-*dihydrolubimin* (**23**), 3,15-*Di*-O-*acetyl*-2-*hydroxy*-15-*dihydrolubimin* (**24**) *and* 2,3,15-*Tri*-O-*acetyl*-3-*hydroxy*-15-*dihydrolubimin* (**25**).—Treatment of (**20**) (19.0 mg) with 200 mm^3 Ac_2O and 250 mm^3 pyridine in 3.5 cm^3 benzene for 4 h at room temperature followed by TLC (solvent A) afforded (**23**) (crystalline, 9.7 mg), R_f 0.44; (**24**) (oil, 10.4 mg), R_f 0.30; both *m/z* 338 (M^+), 320 ($M - H_2O$)$^+$, 278 ($M - C_2H_4O_2$)$^+$, 260 ($M - C_2H_4O_2 - H_2O$)$^+$, 218 ($M - 2C_2H_4O_2$)$^+$, 200 ($M - 2C_2H_4O_2 - H_2O$)$^+$, and (**25**) (oil, 5.5 mg), R_f 0.63; *m/z* 380 (M^+), 320 ($M - C_2H_4O_2$)$^+$, 302 ($M - C_2H_4O_2 - H_2O$)$^+$, 260 ($M - 2C_2H_4O_2$)$^+$, 242 ($M - 2C_2H_4O_2 - H_2O$)$^+$, 200 ($M - 3C_2H_4O_2$)$^+$.

3,15-*Di*-O-*acetyl*-2-*oxo*-15-*dihydrolubimin* (**32**) *and* 10-epi-3,15-*Di*-O-*acetyl*-2-*oxo*-15-dihydrolubimin (**33**).—Treatment of (**24**) (32 mg) with pyridinium chlorochromate (29.9 mg, 1.5 mol equiv.) in CH_2Cl_2[9] overnight followed by TLC (solvent A) gave (**32**) and (**33**) (97:3, oil, 23.4 mg), R_f 0.55; *m/z* 336 (M^+), 294, 276 ($M - C_2H_4O_2$)$^+$, 234, 216 ($M - 2C_2H_4O_2$)$^+$ and unchanged (**24**) (3.3 mg).

2,15-*Di*-O-*acetyl*-3-*oxo*-15-*dihydrolubimin* (**34**).—Oxidation of (**23**) (8.23 mg) with Jones' reagent and TLC (solvent A) gave (**34**) (oil, 6.7 mg), R_f 0.57; *m/z* 336 (M^+), 294, 276 ($M - C_2H_4O_2$)$^+$, 234, 216 ($M - 2C_2H_4O_2$)$^+$.

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