Determination of the Absolute Configuration of Hydroxyiridals by Chiroptical and NMR Spectroscopic Methods

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Hydroxyiridals – monocyclic triterpenoids – are found abundantly in sword lilies. They feature a homofarnesyl side chain that is hydroxylated at one of the allylic methylene groups, which gives rise to a chiral carbon atom. The absolute configuration of six hydroxyiridals from different sources was determined by the exciton chirality method and by NMR spectroscopy of the diastereoisomeric MTPA and MPA esters. All three measurements gave consistent results, establishing the (*R*) configuration for all of the hydroxyiridals. The reliability of these methods for the stereochemical analysis of acyclic secondary allylic alcohols was confirmed by applying them to a synthetic model compound of known configuration. In the course of this study, two new iridals – 16,26-dihydroxyiridal **7** and 18,19-epoxyiridal **8** – were isolated from extracts of *I. versicolor* and the iridal fraction of *I. missouriensis* was analyzed thoroughly.

Introduction

The known hydroxyiridals **1**−**6** are a subfamily of the more than 30 different iridals, which have been found in lipid extracts of various sword lilies.[1] They are monocyclic triterpenoids featuring a homofarnesyl side chain that is hydroxylated at one of the allylic methylene groups. The configuration of the typical six-membered iridal ring system has been determined to be (6*R*,10*S*,11*S*) (the carbon skeleton of the iridals is numbered in analogy to squalene) by X-ray analysis and chemical degradation,[2,3] and this stereochemistry has been assigned to all iridals known to date based on degradation reactions, physical data and biosynthetic considerations.[1,4,5]

Only in one recent case has the chirality of one of the allylic alcohols in the terpenoid chain been studied. Yoshida et al.[5] determined the (16*R*) configuration of 16-hydroxyiridal **1** from *Belamcanda chinensis* by NMR spectroscopy, using the (*R*)- and (*S*)-methoxy(trifluoromethyl)phenylacetic acid (MTPA) esters, according to the modified Mosher method.[6] It is apparent in the literature that this method is the most popular one for the stereochemical analysis of secondary alcohols, because it gives information about both the stereochemistry and the enantiomeric purity of these compounds. It has been used also for several natural products with terpenoid chains resembling the hydroxyiridals.[7,8] So far, however, the validity of the method for acyclic systems has not been evaluated, and its reliability is not undisputed. Thus, Riguera et al.[9] recommend the use of methoxyphenylacetic acid (MPA) in place of MTPA, because a much more certain prediction of the absolute stereochemistry becomes possible as a result of both the smaller number of possible conformers of the resulting esters and the larger values of Δδ of signals in their ^{1}H NMR spectra. The chirality of secondary allylic alcohols can be elucidated also with the exciton chirality method, which has been investigated thoroughly for acyclic systems, and relies on the fact that benzoates of these alcohols show a Cotton effect in their CD spectra, the sign of which can be connected directly to the absolute configuration of the alcohol.[10] Although very reliable, this method has been used rarely, presumably because in many cases other chromophores are present in the molecule of interest, which may preclude the determination of an unambiguous result.

We report here on the use of all three of these methods for the stereochemical analysis of the hydroxyiridals, and reconfirm their reliability by applying them to a synthetic model compound of known absolute configuration.

Results and Discussion

The most common hydroxyiridal is 16-hydroxyiridal **1**, which has been present in all *Iris* extracts studied to date.[1] From a previous separation of an *I. sibirica* extract,[11] we had this natural product at hand in sufficient quantity for the current analyses. The other terpenoids were isolated from lipid extracts of *Iris* rhizomes according to a procedure outlined previously.[12] We have shown some time ago

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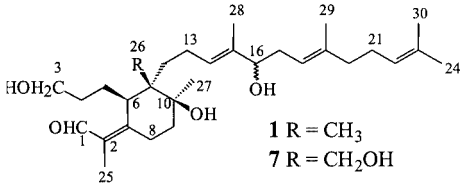
that *I. versicolor* is a rich source of 17,26-dihydroxyiridal **2**[11] and, therefore, we obtained this compound by extracting rhizomes of this plant once again. Besides **2**, this separation yielded also enough 17-hydroxyiridal **3** for the derivatization reactions.

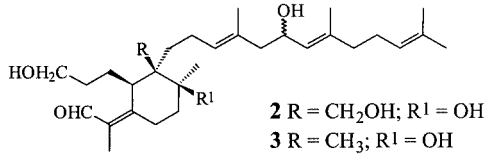
In addition, two hitherto unknown iridals were found unexpectedly and their structures elucidated by spectroscopic methods (UV, MS, 1D and 2D NMR). Most resonances in the ^1H and ^{13}C NMR spectra of the major compound correlated well with those of 16-hydroxyiridal **1**, except for the missing methyl group at C-26. Instead, in the ^1H NMR spectrum an AB system of a CH_2O group was present and centered at $\delta_H = 3.98$ ppm, with the corresponding ^{13}C NMR signal observed at $\delta_C = 68.3$ ppm. The presence of an additional hydroxy group was confirmed by the electrospray mass spectrum, which showed a quasimolecular ion peak $[M + Na]^+$ at $m/z = 513$. Thus, the compound is the 16,26-dihydroxyiridal **7**.

A quasimolecular ion peak $[M + H]^+$ at $m/z = 475$ in the mass spectrum of the minor component, in conjunction with the NMR spectra, indicated the molecular composition to be $C_{30}H_{50}O_4$. Signals of five oxygenated carbon atoms, however, were seen in the ^{13}C NMR spectrum, of which three were assigned to the iridal ring system. The remaining two C−O resonances were assigned to a trisubstituted oxirane ring system, which showed signals for the quaternary carbon atom at $\delta_C = 60.8$ ppm and for the methine group at $\delta_C = 63.3$ ppm ($\delta_H = 2.65$ ppm). Detailed analysis of 2D NMR spectra showed that the epoxide ring is located in the terpenoid side chain and the natural product was identified as 18,19-epoxyiridal **8**. The NMR spectroscopic data of **8** closely resemble the signals of the homologous 18,19-epoxy-10-deoxyiridal **9**, which has been isolated recently from *I. germanica* Rococo.[13] Based on the physical data of **7** and **8** and the earlier investigations,[1,4,5] (6*R*,10*S*,11*S*) configurations again can be assigned to both compounds.

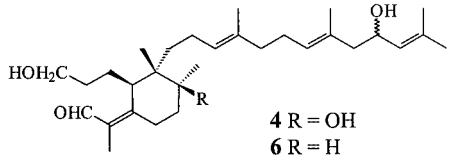
21-Hydroxyiridal **4** is by far the main iridal in extracts from *I. variegata*[1] and, therefore, was obtained from this plant. Finally, after a check of our extracts in stock, *I. missouriensis* was chosen as the source of 10-deoxy-17-hydroxyiridal **5**. This plant has been studied once before, when 16-hydroxyiridal **1** was isolated in small amounts from a petroleum ether extract.[14] In a $CHCl_3$/MeOH extract, however, we identified several iridals by LC/UV, LC/MS, and comparison with authentic standards. The main components of the iridal fraction are 26-hydroxyiridal **10** (44%) and the (13*R*)-hemiacetal **11a** (24%). As side products, we identified **1** (1%), **2** (5%), **3** (5%), **7** (2%), iridal **12** (7%), and the 28-hydroxyspirotriene **13** (6%). Besides **5**, some 17-hydroxyiridal **3** also was isolated from this extract. The only compound that could not yet be isolated in sufficient quantity for the stereochemical analysis was 10-deoxy-21-hydroxyiridal **6**, obtained originally from *I. germanic*a.[15]



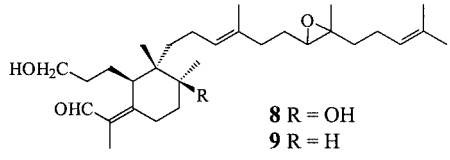
1 R = CH_3
7 R = CH_2OH



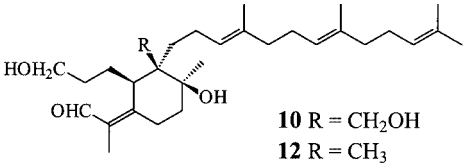
2 R = CH_2OH; R^1 = OH
3 R = CH_3; R^1 = OH
5 R = CH_3; R^1 = H



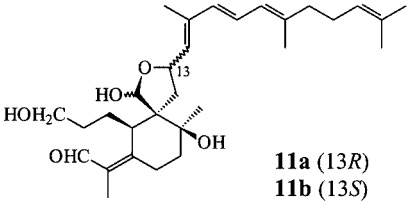
4 R = OH
6 R = H



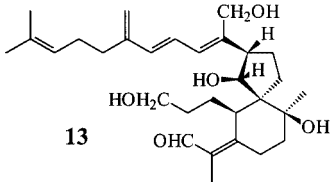
8 R = OH
9 R = H



10 R = CH_2OH
12 R = CH_3



11a (13*R*)
11b (13*S*)



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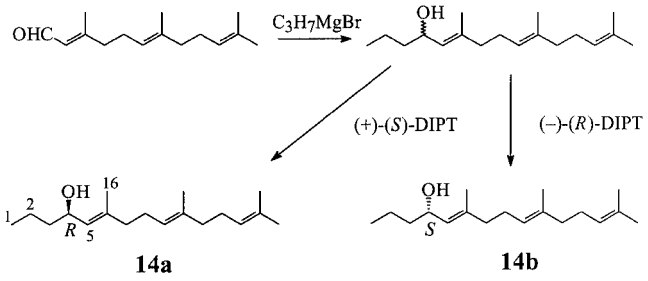
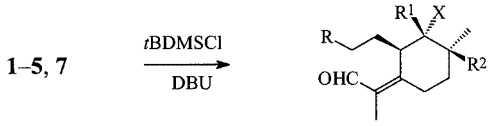
The model compounds (4*R*)- and (4*S*)-hydroxy-6,10,14-trimethylpentadeca-5,9,13-trienes (**14a** and **b**, respectively) were synthesized as outlined in Scheme 1, utilizing Sharpless epoxidation[16] as the decisive step for the separation of enantiomers and the definition of their absolute configurations.

All three methods for the stereochemical analysis are based on the esterification of the chiral secondary hydroxy groups. Therefore, it was necessary to protect the primary

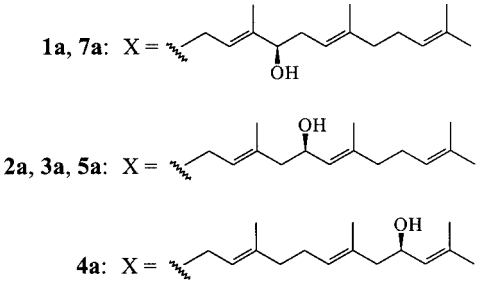


Scheme 1. Synthesis of the (4*R*)- and (4*S*)-hydroxy-6,10,14-trimethylpentadeca-5,9,13-trienes (**14a** and **14b**)

hydroxy groups at C-3 and, if applicable, C-26 of the hydroxyiridals, in order to avoid their interference in this reaction or in the spectroscopic determination of the absolute configuration. Since the CD method demands a protecting group lacking a chromophore, the *tert*-butyldimethylsilyl moiety (*t*BDMS) was chosen. Selective silylation of the primary hydroxy groups was achieved easily with *t*BDMSCl and diazabicycloundecene (DBU) as catalyst,[17] to yield the silylated products **1a**−**5a** and **7a** (Scheme 2).

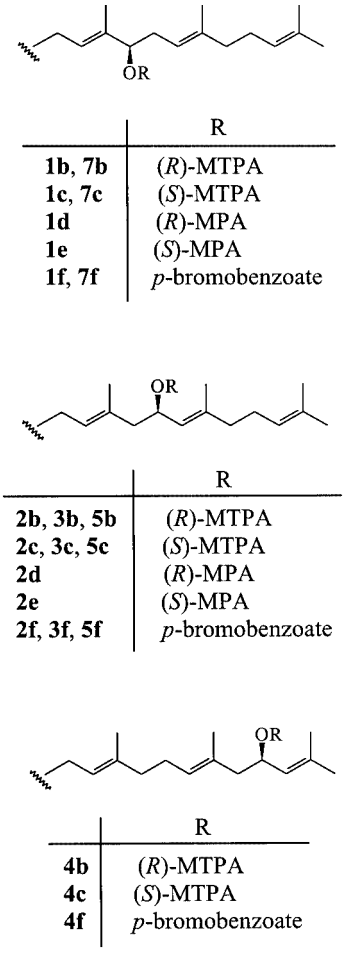


	R	R^1	R^2
1a	$CH_2OSi(CH_3)_2tBu$	CH_3	OH
2a	$CH_2OSi(CH_3)_2tBu$	$CH_2OSi(CH_3)_2tBu$	OH
3a	$CH_2OSi(CH_3)_2tBu$	CH_3	OH
4a	$CH_2OSi(CH_3)_2tBu$	CH_3	OH
5a	$CH_2OSi(CH_3)_2tBu$	CH_3	H
7a	$CH_2OSi(CH_3)_2tBu$	$CH_2OSi(CH_3)_2tBu$	OH



Scheme 2. Silylation of the primary alcohol function(s) of hydroxyiridals **1**−**5** and **7**

Subsequently, the (*R*)- and (*S*)-Mosher esters of the secondary alcohols **1b/c**−**5b/c** and **7b/c** were prepared by reactions with (+)-(*S*)- and (−)-(*R*)-MTPACl, respectively (Scheme 3).[6] Correspondingly, the protected hydroxyiridals **1a** and **2a** were esterified with both (*R*)- and (*S*)-MPA, according to Trost et al.[18] to give the MPA esters **1d/e** and **2d/e**, respectively. For the exciton chirality studies, the silylated triterpenoids were converted into their *p*-bromobenzoate esters[10] **1f**−**5f** and **7f** and their α,β-unsaturated aldehyde functions were reduced with $NaBH_4$ to remove the iridal chromophore. All products were purified and characterized spectroscopically (MS, UV, 1D and 2D NMR). Since characteristic differences in the NMR spectra generally are found close to the diastereoisomeric esters, a detailed list of NMR spectral resonances of only the MPA esters **1d/e** and the Mosher esters **3b/c**, **4b/c**, and **7b/c**, are given as representative examples in Table 1 (^1H) and Table 2 (^{13}C).



Scheme 3. Esters of the protected hydroxyiridals **1a**−**5a** and **7a**

According to Kakisawa et al., the stereochemistry of the substituents next to the secondary hydroxy group is determined by the differences of their ^1H NMR resonances ($\Delta\delta_{SR} = \delta_S - \delta_R$) in the (*S*)- and (*R*)-MTPA esters.[6] When this analysis is applied to the values of $\Delta\delta_{SR}$ for the hydroxyiridal derivatives listed in Table 3, the (*R*) configuration is found for all carbinol groups in the homofarnesyl side chains of the triterpenoids.

In the diastereoisomeric MPA esters, shielding of the substituents is more important than the deshielding that is the crucial effect in the MTPA derivatives.[9] Hence, if Kakisawa's model is applied, the signs of the values of $\Delta\delta_{SR}$ must be switched. As shown in Table 3, this assumption turns out to be the case for the MPA derivatives **1d/e** and **2d/e**, and corroborates the results obtained with the Mosher esters.

Table 1. ^{1}H NMR chemical shifts (δ) of the (*R*)- and (*S*)-MPA esters **1d/e** and the (*R*)- and (*S*)-MTPA esters **3b/c**, **4b/c** and **7b/c** in $CDCl_3$ (300 MHz) [*J* values (Hz) are given in parentheses]

	1d	1e	3b	3c	4b	4c	7b	7c
1-H	10.10 s	10.14.	10.14 s	10.15 s	10.16 s	10.16 s	10.20 s	10.19 s
3-H	3.54 t (6)	3.55 t (6.5)	3.55 t (6)	3.55 t (6)	3.55 t (6.5)	3.55 t (6.5)	3.54 t (6.5)	3.54 t (6.5)
4-H	1.18/1.31 2m	1.17/1.31 2m	1.2−1.4 m	1.2−1.4 m	1.18−1.30 m	1.18−1.27 m	1.19−1.23 m	1.20−1.25 m
5-H	1.83−1.87 m	1.83−1.87 m	1.93 m	1.93 m	1.93−2.02 m	1.88−1.98 m	1.90−1.96 m	1.90−1.95 m
6-H	3.19 br d (11.6)	3.23 br d (11.6)	3.24 br d (11)	3.25 br d (11)	3.27 brd (11)	3.28 br d (11)	3.48 m	3.48 m
8-H	2.51/2.57 2m	2.51/2.57 2m	2.53m	2.53 m	2.54/2.64 2m	2.53/2.66 2m	2.50/2.69 2m	2.52/2.69 2m
9-H	1.62 m	1.62 m	1.55/1.65 2m	1.56/1.65 2m	1.60/1.82 2m	1.64/1.78 2m	1.64/1.73 2m	1.63/1.74 2m
12-H	1.10/1.27 2m	1.08/1.27 2m	1.03/1.11 2m	1.03/1.11 2m	1.05−1.29 m	1.10−1.28 m	1.20−1.25 m	1.17−1.27 m
13-H	1.82 m	1.82 m	1.72 m	1.74 m	1.82−1.87 m	1.80−1.90 m	1.80−1.88 m	1.75−1.85 m
14-H	5.04 br t (7)	5.22 br t (7)	4.95 t (7)	5.03 br t (7)	4.92 t (6)	4.93 t (7)	5.32 t (7)	5.25 t (7)
16-H	5.06 m	5.06 br t (6.5)	2.09/2.32 2m	2.13/2.37 2m	1.85 m	1.87 m	5.28 t (7)	5.25 t (7)]
17-H	2.15−2.35 m	2.04−2.25 m	5.81 dt (6,8)	5.79 dt (6,8)	1.91 m	1.98 m	2.22/2.36 2m	2.26/2.40 2m
18-H	4.93 t (7)	4.71 t (7)	5.14 br d (8)	5.03 br d (8)	5.06 t (7)	5.15 t (6.5)	4.88 t (6.5)	4.99 t (7)
20-H	1.90 m	1.77 m	2.02 m	1.98 m	2.14/2.34 2m	2.19/2.40 2m	1.85−1.95 m	1.91−2.00 m
21-H	1.97 m	1.89 m	2.04 m	2.01 m	5.82 ddd (2,9,14)	5.80 ddd (2,9,14)	1.96−2.03 m	1.97−2.04 m
22-H	5.04 br t (6)	5.00 brt (6)	5.04 br t (7)	5.01 br t (7)	5.16 br d (9)	5.01 brd (9)	5.04 t (6.5)	5.05 t (7)
24-H	1.66 s	1.66 s	1.65 s	1.64 s	1.72 s	1.68 s	1.66 s	1.66 s
25-H	1.80 s	1.81 s	1.81 s	1.81 s	1.82 s	1.82 s	1.82 s	1.82 s
26-H	0.99 s	1.04 s	1.04 s	1.05 s	1.07 s	1.07 s	3.88 AB system (10.3)	3.88 AB system (10.5)
27-H	1.10 s	1.11 s	1.11 s	1.10 s	1.14 s	1.14 s	1.23 s	1.24 s
28-H	1.23 s	1.46 s	1.50 s	1.57 s	1.48 s	1.49 s	1.51 s	1.37 s
29-H	1.56 s	1.43 s	1.73 s	1.72 s	1.54 s	1.61 s	1.51 s	1.56 s
30-H	1.57 s	1.56 s	1.58 s	1.57 s	1.74 s	1.74 s	1.57 s	1.57 s
Phe-2′,6′	7.30−7.44 m	7.30−7.44 m	7.48−7.52 m	7.48−7.52 m	7.48−7.52 m	7.48−7.52 m	7.45−7.50 m	7.45−7.509 m
Phe-3′,4′,5′	7.30−7.44 m	7.30−7.44 m	7.35−7.39 m	7.35−7.39 m	7.34−7.37 m	7.34−7.37 m	7.34−7.37 m	7.34−7.37 m
OCH_3	3.38 s	3.38 s	3.49 s	3.48 s	3.50 s	3.51 s	3.48 s	3.49 s
1′-H	4.69 s	4.71 s						
$(CH_3)_2Si$	0.00 s	0.01 s	0.02 s	0.01 s	0.01 s	0.01 s	C-3: 0.00 s/C-26: 0.14 s	C-3: 0.00 s/C-26: 0.14 s
*t*BuSi	0.86 s	0.86 s	0.87 s	0.87 s	0.87 s	0.87 s	C-3: 0.86 s/C-26: 0.92 s	C-3: 0.86 s/C-26: 0.93 s

The same stereochemical assignment also was found when Nakanishi's exciton chirality method[10] was used, since in their CD spectra all the *p*-bromobenzoyl esters exhibited − after reduction of the iridal chromophores − negative Cotton effects in the region 240−260 nm (Table 4). It is worth mentioning that this Cotton effect was seen also in the non-reduced *p*-bromobenzoates (Table 4), but shifted to slightly lower wavelengths. In addition, a second, positive CD is observed near 254 nm that certainly is due to the unsaturated aldehyde, which is located next to three chiral centers at C-6, C-10 and C-11. Similar CD spectra with a positive Δε have been recorded for 16-hydroxyiridal **1** and several of its derivatives and have been interpreted as an indication of the (6*R*,10*S*,11*S*) stereochemistry of the iridal ring.[5]

When applied to the (*R*) and (*S*) model compounds **14a/b**, consistent corresponding results were obtained with all three analytical procedures (data in the Exp. Sect.). Thus, their reliability as methods for the stereochemical analysis of acyclic secondary alcohols was confirmed.

The NMR spectra of the diastereoisomeric MTPA or MPA esters suggested that all the hydroxyiridals are enantiomerically pure. This observation does not exclude the possibility that the epimeric secondary alcohols exist, but as diastereoisomers [on the prerequisite of a uniform (6*R*,10*S*,11*S*) configuration] one would expect them to show different chromatographic behavior and, therefore, to have been separated during the isolation. None of these compounds is known yet, but there is at least one indication that their occurrence might be likely. Belachinal (**11b**), isolated from *Belamcanda chinensis*[5] and also found in *I. fulva*,[19] is (13*S*)-configured and is separated easily by HPLC from its more common (13*R*) epimer **11a**. The hemiacetal ring of both compounds presumably is formed by cyclization of a C-26 aldehyde with a C-13 carbinol, a reaction that should proceed without epimerization at C-13. Therefore, it is to be expected that the corresponding 13-hydroxyiridals are biosynthetic intermediates. Since all alcohols examined in this study are (*R*)-configured, independent of their origin, and since the (13*R*)-hemiacetal **11a** and its (*S*) epimer **11b** have not been found within the same plant extract, the enzymatic oxygenation of the homofarnesyl chain seems to be highly stereospecific.

The chiroptical and NMR methods used for these stereochemical analyses both work well for the problem at hand, and both have their advantages and disadvantages. The CD spectroscopy requires less material, but an unambiguous result can be expected only when an additional reaction step is carried out − namely, the reduction of the iridal chromophore. With the hydroxyiridals examined in this study it is a fortunate coincidence that the Cotton effects of the iridal moiety and the *p*-bromobenzoate unit possess opposite signs. More material is needed for the NMR analysis, but on the other hand it provides the stereochemistry as well as giving information on the enantiomeric purity of the com-

Table 2. ^{13}C NMR chemical shifts (δ) of the (*R*)- and (*S*)-MPA esters **1d/e** and the (*R*)- and (*S*)-MTPA esters **3b/c**, **4b/c** and **7b/c** in $CDCl_3$ (75 MHz)

	1d	**1e**	**3b**	**3c**	**4b**	**4c**	**7b**	**7c**
C-1	189.9	190.1	189.9	189.9	190.0	190.0	189.8	189.8
C-2	133.0	133.3	133.1	133.1	133.0	132.7	133.0	133.0
C-3	63.0	63.4	63.0	63.0	62.9	62.9	63.0	63.0
C-4	32.2	32.3	29.2	29.2	32.7	32.3	32.5	32.4
C-5	26.2	26.3	26.0	26.0	26.0	26.0	26.7	26.6
C-6	42.5	43.5	43.0	43.0	42.5	42.5	42.5	42.4
C-7	162.7	163.5	162.6	162.6	163.6	163.3	162.6	162.6
C-8	23.5	23.7	25.7	25.7	22.9	22.9	24.3	24.2
C-9	36.5	36.6	37.1	37.1	36.6	37.0	36.7	36.6
C-10	74.6	74.6	74.5	74.5	75.1	74.6	75.3	75.3
C-11	44.1	44.4	44.2	44.2	44.4	44.1	45.7	45.7
C-12	31.2	31.4	32.2	32.2	32.9	32.8	32.9	32.9
C-13	21.7	21.6	22.2	22.2	22.1	22.0	21.0	20.9
C-14	128.1	128.1	128.1	128.1	124.1	124.1	129.6	129.0
C-15	132.2	133.3	129.7	129.4	135.4	135.0	132.2	132.2
C-16	79.5	79.8	44.1	44.1	38.9	38.6	82.0	82.0
C-17	30.9	30.6	72.1	72.3	26.0	26.0	39.1	39.1
C-18	119.0	118.5	122.3	121.8	128.0	128.0	118.2	118.2
C-19	137.9	137.6	142.0	142.0	130.0	129.8	138.0	138.0
C-20	39.1	39.1	39.2	38.9	44.1	44.1	39.2	39.2
C-21	26.2	26.2	26.0	26.1	72.3	72.3	25.3	25.2
C-22	123.9	123.7	123.1	123.4	122.5	122.5	124.0	124.0
C-23	131.4	131.5	131.4	131.4	138.5	138.3	131.3	131.3
C-24	25.2	25.4	25.2	25.2	25.2	25.3	25.2	25.2
C-25	10.3	10.8	10.2	10.4	10.3	10.3	10.3	10.3
C-26	16.9	17.7	17.0	16.9	17.4	17.4	68.8	68.8
C-27	25.9	26.3	25.9	25.6	26.0	26.0	26.0	26.0
C-28	11.1	11.6	15.2	15.1	15.0	15.1	10.4	10.4
C-29	16.1	15.9	16.1	16.0	15.8	15.8	16.1	16.1
C-30	17.7	17.6	17.1	17.4	18.2	18.2	16.9	16.7
Phe-2′,6′	127.2	127.2	127.3	127.3	128.1	128.1	127.3	127.3
Phe-3′,4′,5′	128.8	128.8	128.3	128.4	128.9	128.9	128.1	128.1
OCH_3	57.3	57.3	55.1	55.0	55.0	55.1	55.0	55.1
C-1′	82.0	82.0						
$(CH_3)_2Si$	−5.6	−5.6	−6.2	−6.2	−5.3	−5.3	C-3: −6.1 C-26: − 6.1	C-3: −6.1 C-26: −6.1
*t*BuSi-CH_3	25.4	25.4	25.2	25.3	26.0	26.0	C-3: 25.2 C-26: 25.2	C-3: 25.2 C-26: 25.2
*t*BuSi-C	18.3	18.3	18.3	18.3	18.5	18.5	C-3: 18.4 C-26: 18.4	C-3: 18.4 C-26: 18.4

Table 3. Values of $\Delta\delta_{SR}$ (Hz) for selected protons in the ^1H NMR spectra (300 MHz, $CDCl_3$) of the MTPA and MPA derivatives **1b/c**−**5b/c** and **7b/c**

	MTPA Derivatives						MPA Derivatives	
	1b/c	**2b/c**	**3b/c**	**4b/c**	**5b/c**	**7b/c**	**1d/e**	**2d/e**
14-H	−21	+36	+24		+18	−21	+54	−51
16-H		+18	+15	+6	+12			−33
17-H	+15			+21		+12	−33	
18-H	+33	−45	−33	+27	−45	+33	−66	+36
20-H	+12	−12	−12	+18	−15	+15	−39	+36
21-H	+12	−9	−9		−15	+3	−24	
22-H	+3	−9	−9	−45	−9	+3	−12	+18
24-H				−12				
28-H	−51	+15	+21		+30	−42	+69	−30
29-H	+12	−3	−3	+21	−3	+15	−39	+30

Table 4. CD data (MeOH) for the *p*-bromobenzoates of **1f**−**5f** and **7f** after and before reduction of the α,β-unsaturated aldehyde function

	Reduced		Unreduced	
	λ_{ext} (nm)	Δε	λ_{ext} (nm)	Δε
1f	260.9	−2.52	nd	nd
2f	251.2	−2.90	240.8	−1.98
			260.5	+7.52
3f	248.5	−1.80	247.1	−2.20
			260.4	+2.02
4f	245.7	−2.10	241.2	−1.69
			261.5	+1.30
5f	245.8	−1.74	242.2	−1.18
			261.3	+1.30
7f	261.5	−2.60	233.7	−1.20
			256.4	+7.02

pound. In concert with prior studies,[9] the NMR spectroscopic data of MPA esters are more clearly interpretable than are the data of their MTPA counterparts, since they show much larger values of $\Delta\delta_{SR}$. Moreover, we found that the hydroxyiridals react with MPA faster, under milder conditions, and with better yields, than with MTPACl. Therefore, the former seems to be the method of choice.

Experimental Section

General Remarks: MPLC: Büchi model 681 chromatography pump, columns 240 mm, 20 mm i.d., RP 18 (14−40 μm). CC: Silica gel 60, 70−230 mesh, Merck. Flash CC: Silica gel 32−63, ICN. Analytical HPLC: Kontron model 200, column LiChrocart RP 18 (125 mm, Merck); solvent MeOH/H_2O, 7:3 (5 min), linear gradient to 100% MeOH (15 min), 100% MeOH (10 min), flow 1 mL/min; Hewlett−Packard 1040A diode-array detector. UV: Spectra were recorded during the HPLC run. HPLC/MS: ThermoFinnigan LCQ equipped with Hewlett−Packard 1100 HPLC and APCI or ESI ion source. Optical rotation: Zeiss 0.05 precision polarimeter. NMR: Bruker AM300 (^{1}H: 300 MHz, ^{13}C: 75 MHz). CD: Jasco model J-715 CD spectrophotometer, solvent MeOH (20 °C).

Plant Material: *I. variegata* was raised from seeds, obtained from the Botanical Garden in Wuppertal, Germany, in 1986. This species and *I. versicolor*, originally used in our study of its constituents,[11] are grown in the author's (F. J. M.) garden. Rhizomes of *I. missouriensis* were collected near White River, north of Lake Superior, Ontario, Canada, in August 1988 and extracted shortly thereafter. The extract was stored in a freezer at −20 °C. According to HPLC analysis the iridal composition remained unchanged during this time.

Extraction and Isolation of the Iridals: The general procedure for the extraction of the rhizomes and the isolation of the natural products by reverse-phase MPLC has been described elsewhere.[12] The crude extract of *I. variegata* (0.59 g) yielded **4** (28 mg), and from the *I missouriensis* extract (2.2 g) both **5** (42 mg) and **3** (41 mg) were isolated. 16-Hydroxyiridal **1** (40 mg) was in stock from a previous separation of an *I. sibirica* extract.[11] From an extract of *I. versicolor* (6 g) we obtained **2** (270 mg), **3** (102 mg), **7** (59 mg) and **8** (7 mg).

(6*R*,10*S*,11*S*,16*R*)-16,26-Dihydroxyiridal (7): UV: λ_{max} = 254 nm. $[\alpha]_{546}^{21}$ = +22.5 (*c* = 0.1, MeOH). MS (ESI, MeOH, 1 mM NaOAc/HOAc): *m*/*z* = 513 [M + Na^+]. LC/MS (APCI): *m*/*z* = 473 [M − H_2O + H^+], 455 [M − 2 H_2O + H^+]. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.15−1.30 (m, 2 H, 12-H), 1.28 (m, 2 H, 4-H), 1.29 (s, 3 H, 27-H), 1.52 (s, 3 H, 28-H), 1.58 (s, 3 H, 30-H), 1.60 (s, 3 H, 29-H), 1.65/1.80 (2 m, 2 H, 9-H), 1.66 (s, 3 H, 24-H), 1.82 (s, 3 H, 25-H), 1.84−1.92 (m, 2 H, 13-H), 2.01−2.13 (m, 2 H, 20-H), 2.05−2.25 (m, 2 H, 5-H), 2.10−2.33 (m, 2 H, 17-H), 2.15−2.26 (m, 2 H, 21-H), 2.52/2.68 (2 m, 2 H, 8-H), 3.54 (m, 1 H, 6-H), 3.57 (m, 2 H, 3-H), 3.90 [t, *J*(16,17) = 6 Hz, 1 H, 16-H], 3.92/4.05 [AB system, J_{AB} = 11.5 Hz, 2 H, 26-H], 5.04 [t, *J*(18,17/22,23) = 6.8 Hz, 2 H, 18-H/22-H], 5.19 [t, *J*(14,13) = 6.8 Hz, 1 H, 14-H], 10.18 (s, 1 H, 1-H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 11.0 (q, C-25), 11.9 (q, C-28), 16.3 (q, C-29), 17.7 (q, C-30), 21.0 (t, C-13), 23.9 (t, C-8), 26.2 (t, C-5), 26.4 (q, C-24), 26.5 (q, C-27), 27.1 (t, C-21), 32.1 (t, C-12), 34.2 (t, C-17), 35.4 (t, C-4), 37.1 (t, C-9), 39.8 (t, C-20), 42.6 (d, C-6), 46.7 (s, C-11), 62.5 (t, C-3), 68.3 (t, C-26), 76.1 (s, C-10), 76.7 (d, C-16), 119.9 (d, C-18), 124.1 (d, C-22), 125.1 (d, C-14), 131.6 (s, C-23), 133.1 (s, C-2), 137.3 (s, C-15), 138.8 (s, C-19), 162.6 (s, C-7), 190.1 (d, C-1) ppm.

(6*R*,10*S*,11*S*)-18,19-Epoxyiridal (8): UV: λ_{max} = 254 nm. $[\alpha]_{546}^{21}$ = +23.7 (*c* = 0.1, MeOH). LC/MS (APCI): *m*/*z* = 475 [M + H^+], 457 [M − H_2O + H^+]. ^{1}H NMR ($CDCl_3$, 300 MHz): 1.07 (s, 3 H, 26-H), 1.14 (s, 3 H, 27-H), 1.22 (s, 3 H, 29-H), 1.30−1.10 (m, 2, 12-H), 1.40−1.20 (m, 2 H, 4-H), 1.52 (s, 3 H, 28-H), 1.57 (m, 2 H, 17-H), 1.59 (s, 3 H, 30-H), 1.60−1.30 (m, 2 H, 20-H), 1.66 (s, 3 H, 24-H), 1.82 (s, 3 H, 25-H), 1.85/1.66 (m, 2 H, 9-H), 1.75−1.85 (m, 2 H, 13-H), 1.90−2.00 (m, 2 H, 5-H), 2.00−2.05 (m, 2 H, 16-H), 2.10−2.00 (m, 2 H, 21-H), 2.60−2.50 (m, 2 H, 8-H), 2.65 [t, *J*(18,17) = 6.5 Hz, 1 H, 18-H], 3.29 [dd, *J*(6,5) = 10.9/2.4 Hz, 1 H, 6-H], 3.59 [t, *J*(3,4) = 6.5 Hz, 2 H, 3-H], 4.99 [t, *J*(14,13) = 6.8 Hz, 1 H, 14-H], 5.06 [t, *J*(22,23) = 6.8 Hz, 1 H, 22-H], 10.16 (s, 1 H, 1-H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 10.9 (q, C-25), 15.9 (q, C-28), 16.5 (q, C-29), 17.7 (q, C-30), 17.9 (q, C-26), 22.1 (t, C-13), 23.9 (t, C-8), 23.9 (t, C-21), 25.7 (q, C-24), 26.3 (q, C-27), 26.6 (t, C-5), 27.1 (t, C-17), 32.7 (t, C-4), 36.2 (t, C-16), 37.0 (t, C-12), 37.2 (t, C-9), 38.8 (t, C-20), 43.4 (s, C-6), 44.7 (d, C-11), 60.8 (s, C-19), 63.0 (t, C-3), 63.3 (d, C-18), 75.0 (s, C-10), 123.7 (d, C-22), 124.4 (d, C-14), 131.8 (s, C-23), 133.1 (s, C-2), 134.6 (s, C-15), 162.8 (s, C-7),190.0 (d, C-1) ppm.

(4*R*)- and (4*S*)-Hydroxy-6,10,14-trimethylpentadeca-5,9,13-triene (14a/b): *n*PrBr (1.3 g, 10.53 mmol) was added dropwise to Mg (0.26 g, 10.7 mmol) in Et_2O (5 mL) and then the solution was heated at 30 °C for 30 min. *all*-(*E*)-Farnesal (1.82 g, 8.3 mmol) in Et_2O (4 mL) was then added and the mixture was kept at 30 °C for 90 min. After hydrolysis with H_2O (10 mL) and extraction with Et_2O, the product was purified by flash CC on silica gel (cyclohexane/Et_2O, 80:20) to give a colorless oil (1.66 g, 72.5%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.91 [t, *J*(1,2) = 7 Hz, 3 H, 1-H], 1.34−1.55 (m, 4 H, CH_2), 1.59 (br s, 6 H, 17-H/18-H), 1.67 (br s, 6 H, 15H/16H), 1.94−2.11 (m, 8 H, CH_2), 4.35 [dt, *J*(4,5) = 7.6, *J*(4,3) = 6.2 Hz, 1 H, 4-H], 5.08 (m, 2 H, 9-H/13-H), 5.15 [d, *J*(5,4) = 7.6 Hz, 1 H, 5-H] ppm. The enantiomers were resolved by Sharpless epoxidation[16] with (+)-(*S*)- and (−)-(*R*)-diisopropyl tartrate, respectively. Ti(O*i*Pr)$_4$ (Aldrich; 27 mg, 0.095 mmol) and diisopropyl tartrate (Aldrich; 21 mg, 0.091 mmol) were added successively to CH_2Cl_2 (5 mL) at −25 °C. After 10 min, the racemic alcohol (0.5 g, 1.89 mmol) in CH_2Cl_2 (2 mL) was added and the solution was stirred for 30 min. Finally, *tert*-butyl hydroperoxide (45% solution in anhydrous CH_2Cl_2; 0.39 mL, 0.38 mmol) was added. The reaction mixture was stirred and its progress monitored by GC. At 55% turnover, the reaction was quenched by the addition of 10% aqueous tartaric acid (5 mL). After 30 min, the solution was warmed to room temperature. The organic layer was washed once with water and concentrated to give a colorless oil, which was dissolved in CH_2Cl_2 (5 mL). After stirring with 1 N NaOH (3 mL) for 30 min, the organic phase was washed with brine, dried (Na_2SO_4) and the product was purified by flash CC on silica gel (cyclohexane/Et_2O, 95:5). Use of (+)-(*S*)-diisopropyl tartrate gave of the alcohol (*R*)-**14a** (0.125 g, 63.5%) as a colorless oil, $[\alpha]_{546}^{20}$ = −15 (*c* = 0.04, $CHCl_3$). The (*S*) enantiomer **14b** (0.1 g, 52.9%) was obtained with (−)-(*R*)-diisopropyl tartrate {colorless oil, $[\alpha]_{546}^{20}$ = +9 (*c* = 0.01, $CHCl_3$)}.

***t*BDMS Derivatives of the Hydroxyiridals 1a−5a and 7a:** In a typical run, the pertinent hydroxyiridal (30 mg, 0.063 mmol), DBU (1.8 mg, 0.012 mmol), triethylamine (12.1 mg, 0.12 mmol), and *t*BDMSCl (10.9 mg, 0.072 mmol; 21.8 mg, 0.144 mmol in the cases of the dihydroxyiridals **2** and **7**), were dissolved in CH_2Cl_2 (0.5 mL). The solution was stirred at room temperature and the reaction was monitored by HPLC. After hydrolysis with saturated $NaHCO_3$ solution and extraction with CH_2Cl_2, the organic phase was washed with saturated NaCl solution and dried ($MgSO_4$). The product was purified by CC on silica gel with a cyclohexane/diethyl ether gradi-

ent to give the selectively protected compounds as glasslike solids. Compound (yield): **1a** (24.5 mg, 66%); **2a** (23.5 mg, 55%); **3a** (22.4 mg, 61%); **4a** (27.1 mg, 73%); **5a** (31.9 mg, 84%); **7a** (30.1 mg, 71%). The products were characterized by MS and NMR spectroscopy (data not shown).

Esterification of 1a−5a and 7a

(i) (*R*)- and (*S*)-MTPA Esters 1b/c−5bc and 7b/c: Et_3N (2.5 mg, 0.025 mmol), dimethylaminopyridine (DMAP; 8 mg, 0.063 mmol), and (+)-(*S*)-MTPACl (10.7 mg, 0.036 mmol) were added to a solution of the protected 16-hydroxyiridal **1a** (10 mg, 0.021 mmol) in CH_2Cl_2 (0.5 mL). The mixture was stirred overnight at room temperature. After hydrolysis, extraction with Et_2O, and evaporation of the solvent, the product was purified by flash CC on silica gel (cyclohexane/Et_2O, 90:10) to give of the MTPA ester (*R*)-**1b** as a colorless oil (10.7 mg, 81%). The MTPA esters (*R*)-**2b**−**5b** and (*R*)-**7b** were prepared in the same way: **2b** (9.7 mg, 73%); **3b** (10.6 mg, 78%); **4b** (10.7 mg, 79%); **5b** (7.8 mg, 57%); **7b** (8.0 mg, 60%). Reaction of the protected hydroxyiridals with (−)-(*R*)-MTPACl according to the same procedure yielded the MTPA esters (*S*)-**1c**−**5c** and (*S*)-**7c**: **1c** (9.1 mg, 65%); **2c** (9.6 mg, 72%); **3c** (8.6 mg, 63%); **4c** (10.0 mg, 73%); **5c** (8.3 mg, 61%); **7c** (7.3 mg, 55%). All products were characterized spectroscopically. MS (ESI, MeOH, 1 mM NaOAc/HOAc): **1b/c**, **3b/c**, **4b/c**: *m/z* = 827 $[M + Na]^+$, 593 $[M + Na - MTPAOH]^+$; **2b/c**, **7b/c**: *m/z* = 957 $[M + Na]^+$, 723 $[M + Na - MTPAOH]^+$; **5b/c**: *m/z* = 811 $[M + Na]^+$, 577 $[M + Na - MTPAOH]^+$. The NMR spectroscopic data of **3b/c**, **4b/c** and **7b/c** are given as representative examples in Table 1 (^1H) and Table 2 (^{13}C).

(ii) (*R*)- and (*S*)-MPA Esters 1d/e and 2d/e: Dicyclohexylcarbodiimide (5.4 mg, 0.026 mmol), DMAP (0.3 mg, 0.0026 mmol), and (−)-(*R*)-MPA (8.6 mg, 0.052 mmol) were added to a solution of the silylated 16-hydroxyiridal **1a** (15.0 mg, 0.026 mmol) in CH_2Cl_2 (0.5 mL). The mixture was stirred for 6 h at room temperature. After hydrolysis, extraction with Et_2O, and evaporation of the solvent, the product was purified by flash CC on silica gel (cyclohexane/Et_2O, 90:10) to give the MPA ester (*R*)-**1d** (14.5 mg, 71%) as a colorless clear oil. In the same fashion, the MPA ester (*R*)-**2d** and − by using (+)-(*S*)-MPA − the MPA esters (*S*)-**1e** and (*S*)-**2e** were prepared: **2d** (16.2 mg, 86%); **1e** (15.8 mg, 81%); **2e** (15.8 mg, 81%). All products were characterized spectroscopically. MS (ESI, MeOH, 1 mM NaOAc/HOAc): **1d/e**: *m/z* = 759 $[M + Na]^+$; **2d/e**: *m/z* = 889 $[M + Na]^+$. The NMR spectroscopic data of **1d/e** are given as representative examples in Table 1 (^1H) and Table 2 (^{13}C).

(iii) *p*-Bromobenzoyl Esters 1f−5f and 7f: A mixture of the protected allylic alcohol **1a** (7.5 mg, 0.013 mmol), *p*-bromobenzoyl chloride (8.6 mg, 0.039 mmol), DMAP (6.3 mg, 0.052 mmol), and Et_3N (1.9 mg, 0.019 mmol), in CH_2Cl_2 (0.25 mL) was stirred at room temperature for 5 h. Hydrolysis, extraction with Et_2O, evaporation of the solvent and flash CC on silica gel (cyclohexane/Et_2O, 95:5) yielded the corresponding *p*-bromobenzoates: **1f** (7.0 mg, 70%); **2f** (9.8 mg, 84%); **3f** (7.1 mg, 66%); **4f** (9.5 mg, 92%); **5f** (7.0 mg, 71%); **7f** (8.8 mg, 75%). MS (ESI, MeOH, 1 mM NaOAc/HOAc): **1f**, **3f**, **4f**: *m/z* = 793/795 $[M + Na]^+$; **2f**, **7f**: *m/z* = 925/927 $[M + Na]^+$; **5f**: *m/z* = 777/779 $[M + Na]^+$.

Reduction of the Iridal Chromophore for CD Spectroscopy: An α,β-unsaturated aldehyde **1f**−**5f** or **7f** (5 mg, 0.01 mmol) was stirred with $NaBH_4$ (2 mg, 0.05 mmol) in dry MeOH (200 μL) at room temperature for 3 h. After hydrolysis and extraction with Et_2O, the organic phase was separated, dried with $MgSO_4$, and the solvents were evaporated. The products were purified by CC on silica gel with Et_2O as eluent, to give the primary alcohols in nearly quantitative yield as colorless oils.

Esterification of the Model Compounds: The model compounds **14a/b** were treated in the same way as described above with (−)-(*R*)- and (+)-(*S*)-MTPACl, (−)-(*R*)- and (+)-(*S*)-MPA and *p*-bromobenzoyl chloride, respectively.

(i) (*R*)- and (*S*)-MTPA Esters: The Mosher esters (7−10 mg, 40−57%) were obtained as colorless oils from **14a** or **14b** (10 mg, 0.038 mmol). MS (ESI, MeOH, 1 mM NaOAc): *m/z* = 503 $[M + Na]^+$. In their ^1H NMR spectra (300 MHz), the following values of $\Delta\delta_{SR}$ (Hz) were found: ester of **14a:** 1-H, +15; 5-H, −36; ester of **14b:** 1-H, −15; 5-H, +36.

(ii) (*R*)- and (*S*)-MPA Esters: Reaction of **14a** or **14b** (10 mg, 0.038 mmol) yielded the respective MPA esters as colorless oils (11−13 mg, 67−79%). MS (ESI, MeOH, 1 mM NaOAc): *m/z* = 435 $[M + Na]^+$. In their ^1H NMR spectra (300 MHz) the following values of $\Delta\delta_{SR}$ (Hz) were found: ester of **14a:** 1-H, −36; 2-H, −60; 5-H, +45; 16-H, +30; ester of **14b:** 1-H, +36; 2-H, +60; 5-H, −45; 16-H, −30.

(iii) *p*-Bromobenzoates: The *p*-bromobenzoates (10−12 mg, 58−63%) were obtained as colorless oils by treating **14a** or **14b** (10 mg, 0.038 mmol). MS (ESI, MeOH, 1 mM NaOAc): *m/z* = 469/471 $[M + Na]^+$. Their CD spectra showed the following maxima: ester of **14a:** λ_{ext} ($\Delta\varepsilon$) = 245.5 (−4.97); ester of **14b:** λ_{ext} ($\Delta\varepsilon$) = 243.9 (+3.17).

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