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ABSOLUTE STEREOCHEMISTRY OF DISIDEIN AND OF TWO NEW RELATED HALOGENATED SESTERTERPENOIDS. TWO-DIMENSIONAL NMR STUDIES AND X-RAY CRYSTAL STRUCTURE

G. Cimino*, S. De Rosa*, S. De Stefano*, R. Puliti*, G. Strazzullo*,
C. A. Mattia† and L. Mazzarella†

*Istituto per la Chimica di Molecole di Interesse Biologico del CNR
Via Toiano n.6, 80072, Arco Felice, Napoli, Italy.† Dipartimento
di Chimica, Università, Via Mezzocannone n.4, 80134, Napoli, Italy

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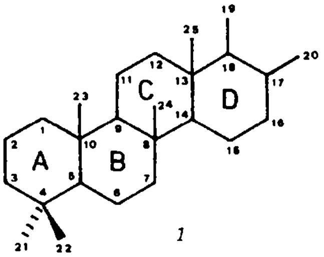
Abstract - Further studies on the marine sponge *Disidea pallescens* gave in addition to the known Disidein (*4*), the unique until now scalarane sesterterpenoid condensed with a hydroxyhydroquinone moiety, two new closely related halogenated sesterterpenoids isolated as triacetates (*6*,*7*). All the ^1H- and ^{13}C-NMR chemical shifts of Disidein triacetate (*5*) were assigned by two-dimensional experiments. Treatment of *5* with chlorine and bromine afforded *6* and *7*, respectively. X ray diffraction analysis of *7* has unambiguously determined the absolute stereochemistry of all these molecules.

Sesterterpenoids showing the carbon skeleton of scalarane (*1*) are frequently present in Porifera of the order Dictyoceratida[1]. Only in two instances these sesterterpenoids were found linked to a hydroxyhydroquinone moiety. The rearranged scalarane sesterterpenoids Toxistylide-A and -B (*2*,*3*) were present in *Microciona toxistyla*[2], while Disidein (*4*) was isolated from *Disidea pallescens*[3]. The structure of *4* was suggested in 1975 without stereochemical implications mainly by the analysis of the mass and ^1H-NMR spectra. Owing to the uniqueness of this carbon skeleton, recently further studies have been prompted in order to obtain a full structural characterization of *4* by 1) an extensive use of the 2D-NMR techniques, 2) the analysis of the minor metabolites of *D. pallescens*, 3) the obtainment of crystalline derivatives of *4* suitable for a resolutive diffractometric analysis.



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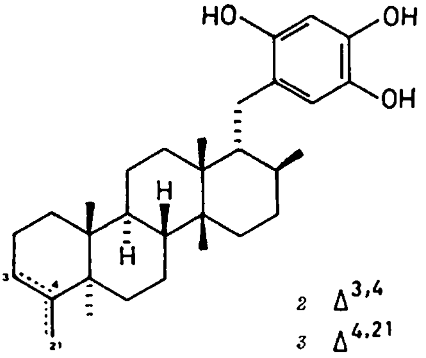
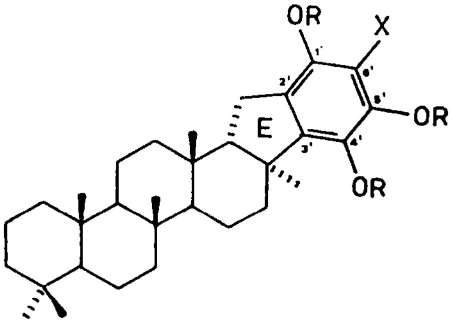


2 $\Delta^{3,4}$
3 $\Delta^{4,21}$

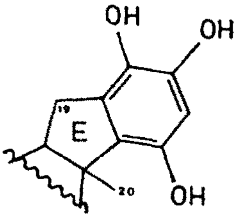


4 R = X = H
5 R = COCH$_3$ X = H
6 R = COCH$_3$ X = Cl
7 R = COCH$_3$ X = Br

TABLE 1 - ^{13}C- and ^{1}H-NMR data for *5*

C	δC^{a}	m^{b}	δH^{c}	δH long range correlated^{d}
1	39.71	t	1.62; 0.80	0.81; 1.40
2	18.20^{e}	t	1.58; 1.40	-
3	42.19	t	1.75; 1.28	1.62; 1.40; 0.83
4	33.26	s	-	0.81; 1.40; 1.58
5	56.96	d	0.81	0.80; 0.83; 1.75
6	18.39^{e}	t	1.58; 1.40	-
7	42.11	t	1.34; 1.10	0.85
8	38.18	s	-	1.80; 1.62; 1.58
9	62.15	d	0.84	1.80; 1.55; 0.85
10	37.69	s	-	1.55; 0.83
11	18.63^{e}	t	1.58; 1.40	-
12	36.79	t	1.55	1.00
13	36.04	s	-	1.80; 1.00
14	52.04	d	1.80	2.08; 1.00; 0.85
15	16.68	t	1.62; 1.32	1.80
16	34.05	t	2.08; 1.82	1.30
17	46.29	s	-	2.08; 1.62; 1.30
18	57.67	d	1.97	1.30; 1.00
19	25.29	t	2.53; 2.43	-
20	25.02	q	1.30	-
21	33.26	q	0.83	1.28; 0.81
22	21.24	q	0.81	0.83
23	15.88	q	0.83	0.84; 0.81
24	18.06	q	0.85	1.80; 0.84
25	23.74	q	1.00	1.80
1'	144.03	s	-	6.78; 2.43
2'	133.34	s	-	6.78; 2.43
3'	148.53	s	-	2.08; 1.30
4'	134.69	s	-	6.78
5'	141.02	s	-	6.78
6'	114.26	d	6.78	-
$-\overset{O}{\overset{\|}{\underline{C}}}-CH_3$	168.14	s		2.28^{g}
"	168.13	s		2.28^{g}
"	161.84	s		2.22^{g}
$-\overset{O}{\overset{\|}{C}}-\underline{C}H_3$	20.70	q	2.28^{f}	
"	20.55	q	2.22^{f}	
"	20.27	q	2.28	

[a] 125.8 MHz, $CDCl_3$. [b] By DEPT sequence. [c] 500 MHz, $CDCl_3$. The assignments were aided by ^{1}H-^{1}H 2D homocorrelation and by direct ^{1}H-^{13}C 2D heterocorrelation. [d] J_{C-H} = 8.3 Hz. [e-g] Signals may be interchanged.



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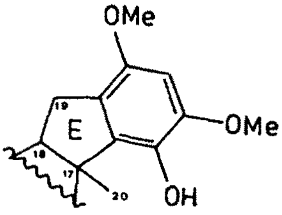

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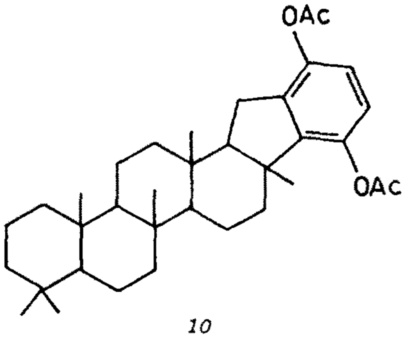

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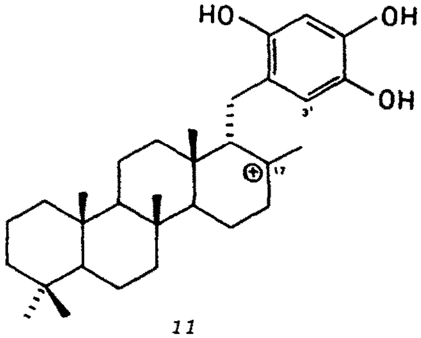

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The n-butanol solubles (16 g) from the acetone extract of *D. pallescens* were obtained following the previously described procedure[3]. After acetylation, the extract was chromatographed on silica gel column yielding in order of increasing polarity an unresolved mixture (200 mg) of sesterterpenoids related to Disidein and, in large amounts, Disidein triacetate (*5*, 6 g).

The aboundance of *5* allowed a detailed spectroscopic study recording a series of mono- and two-dimensional NMR experiments. All the ^1H- and ^{13}C- chemical shifts of *5* were assigned on the basis of the data obtained by dept sequence, ^1H-^1H homo-correlation, direct and long-range ^1H-^{13}C hetero-correlation. The comparison with the reported[4,5] resonances of scalarane-sesterterpenoids strongly supported a *trans-transoid-trans* stereochemistry for the A-D rings of Disidein. In fact, all the carbons of the methyls at the ring-junctions showed diagnostic high-field resonances, in agreement with their axial orientation which, in turn, was confirmed by the expected high-field resonances of C-2, C-6 and C-11. No evidence was obtained on the stereochemistry of the junction of the rings D and E.

Previously[3] the structure *4* was preferred as "the most probable" to the less probable one (*8*, part-structure) on the basis of the observed induced-shifts, in the ^1H-NMR spectra of Disidein dimethylether (*9*, part-structure), of the protons at C-19 and at C-20 after addition of the deuterated lanthanide shift reagent Eu (fod-d_9)$_3$. Now, the alternative structure *8* has been definitively ruled out by the analysis of the long-range ^1H-^{13}C hetero-correlation showing for C-1' and C-2' long range couplings with both the aromatic proton resonating at δ 6.78 (H-6') and the benzylic one at δ 2.43 (H-19). All the NMR data of *5* are reported in Table 1.

The mixture of sesterterpenoids slightly less polar than Disidein was fractionated by μ-porasil HPLC obtaining *6* (80 mg) and *7* (20 mg), along with little amounts (3 mg) of *10* identified only by ^1H-NMR and mass spectrometry. The compounds *6* and *7* show NMR spectra almost identical and strongly reminescent of those of *5*. In the ^1H-NMR spectra of both the new compounds the aromatic proton at δ 6.78 (H-6' in *5*) is absent, while in ^{13}C-NMR spectra of *6* and *7* the doublet at δ 114.26 (C-6' in *5*) is replaced by a singlet at δ 118.90 and δ 109.09, respectively. This spectroscopic evidence and the presence of halogens in the elemental composition of *6* ($C_{37}H_{51}O_6Cl$) and *7* ($C_{37}H_{51}O_6Br$) strongly supported the suggested structures,easily confirmed by treatment of *5* with chlorine or bromine.

Crystallization of *7* from methanol gave crystals suitable for a conclusive X-ray crystallographic analysis which led without ambiguities to determine structure and absolute stereochemistry of all these sesterterpenoids.

In Fig. 1 is reported a perspective drawing of the final X-ray model for the Br-compound *7* showing the complete stereochemistry of the molecule. The absolute configuration derived by the X-ray analysis is in agreement with the configuration assigned to the biogenetically related derivatives of Scalarin on the basis of CD spectra[4]. The sesterterpenoid skeletal structure consists of four hexacyclic rings and one pentacyclic ring *trans* fused. The pentacyclic ring is condensed with the hydroxyhydroquinone system which presents the halogen substituent in C6' position. The values of the bond lengths and angles are reported in Table 3 and on average they compare well with those found for several similar compounds. The mean values of C(sp^3)-C(sp^3), C(sp^3)-C(sp^2) and C(phenyl)-C(phenyl) bonds are respectively 1.546, 1.504 and 1.384 Å. The average value for the C(sp^2)-O, C(phenyl)-O and C=O bond lengths are 1.360, 1.400 and 1.175 Å, respectively.

The presence in the molecule of four axial methyl groups, which protrude from the same side of the ring-fused system, gives rise to skeletal distorsions similar to those found by Croft *et al.* (1983)[6]. The increase of the bond lengths along the "spine" of molecule [defined by C(4),C(5),C(10),C(9),C(8),C(14),C(13),C(18)],and of the bond angles C(4)-C(5)-C(10), C(8)-C(9)-C(10) and C(8)-C(14)-C(13) (see Table 3)

TABLE 2 - Positional parameters and equivalent isotropic temperature factors (Å²) with esd in parentheses for compound *7*

$$B_{eq} = \frac{4}{3} \Sigma_i \Sigma_j b_{ij} \vec{a}_i \vec{a}_j$$

Atom	x	y	z	B_{eq}	Atom	x	y	z	B_{eq}
Br'	0.10322(3)	0.01134(3)	0.08106(2)	6.26(1)	C(16)	0.3592(2)	0.2659(2)	-0.2028(2)	4.59(6)
O(1')	0.3238(2)	-0.0066(2)	0.0571(1)	5.53(5)	C(17)	0.3626(2)	0.1655(2)	-0.1702(2)	4.29(6)
O(2')	0.3643(2)	0.1215(2)	0.1214(2)	6.93(7)	C(18)	0.4579(2)	0.1578(2)	-0.1250(2)	4.25(6)
O(3')	0.1445(2)	0.1989(2)	-0.1682(1)	5.15(5)	C(19)	0.4421(3)	0.0768(3)	-0.0696(2)	4.97(7)
O(4')	0.1310(3)	0.3316(2)	-0.1026(2)	8.41(9)	C(20)	0.3477(3)	0.0894(3)	-0.2323(2)	5.29(7)
O(5')	0.0385(2)	0.1413(2)	-0.0447(1)	5.82(6)	C(21)	0.6865(6)	0.2339(5)	-0.5823(3)	10.5(2)
O(6')	-0.0044(3)	0.0294(3)	-0.1232(3)	11.70(9)	C(22)	0.8436(4)	0.2619(5)	-0.5218(3)	10.1(1)
C(1)	0.7667(4)	0.0540(3)	-0.3991(3)	7.8(1)	C(23)	0.8394(3)	0.1998(4)	-0.3467(3)	7.46(9)
C(2)	0.8291(4)	0.0489(4)	-0.4701(3)	10.3(1)	C(24)	0.6952(3)	0.3182(3)	-0.2499(2)	5.68(8)
C(3)	0.7772(5)	0.0983(4)	-0.5357(3)	9.4(1)	C(25)	0.6238(3)	0.2143(3)	-0.1088(2)	5.73(8)
C(4)	0.7533(4)	0.2013(4)	-0.5186(2)	7.7(1)	C(1')	0.2791(3)	0.0496(2)	0.0023(2)	4.88(7)
C(5)	0.6976(3)	0.2036(3)	-0.4434(2)	5.67(7)	C(2')	0.3341(3)	0.0865(2)	-0.0545(2)	4.72(6)
C(6)	0.6624(3)	0.3026(3)	-0.4229(2)	5.83(8)	C(3')	0.2896(2)	0.1401(2)	-0.1107(2)	4.44(6)
C(7)	0.5860(3)	0.2984(2)	-0.3602(2)	5.02(7)	C(4')	0.1915(3)	0.1547(2)	-0.1081(2)	4.71(6)
C(8)	0.6227(2)	0.2503(2)	-0.2884(2)	4.41(6)	C(5')	0.1362(3)	0.1187(3)	-0.0503(2)	5.07(7)
C(9)	0.6653(2)	0.1534(2)	-0.3120(2)	4.67(6)	C(6')	0.1802(3)	0.0649(2)	0.0051(2)	4.83(6)
C(10)	0.7442(3)	0.1553(3)	-0.3752(2)	5.65(8)	C(7')	0.3650(3)	0.0401(3)	0.1164(2)	5.31(7)
C(11)	0.6896(3)	0.0922(3)	-0.2439(2)	5.90(8)	C(8')	0.4092(3)	-0.0290(3)	0.1693(2)	7.24(9)
C(12)	0.6004(3)	0.0758(2)	-0.1933(2)	5.43(7)	C(9')	0.1148(3)	0.2896(3)	-0.1584(2)	6.00(8)
C(13)	0.5556(2)	0.1683(2)	-0.1667(2)	4.49(6)	C(10')	0.0641(4)	0.3224(4)	-0.2268(3)	8.82(9)
C(14)	0.5332(2)	0.2269(2)	-0.2384(2)	4.17(5)	C(11')	-0.0259(3)	0.0912(4)	-0.0836(3)	7.65(9)
C(15)	0.4622(2)	0.3058(2)	-0.2198(2)	4.57(6)	C(12')	-0.1263(4)	0.1288(5)	-0.0673(5)	10.7(2)

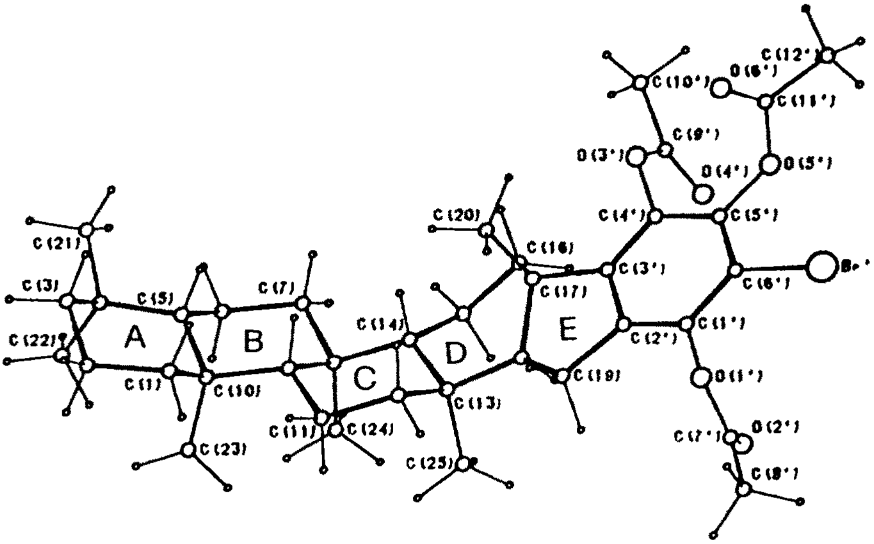

Fig. 1 - Perspective drawing of *7* in correct absolute configuration. Labels for H-atoms and few C-atoms have been omitted for clarity.

TABLE 3 - Bond distances, bond angles and some torsion angles with their esd in parentheses for compound *7*

Bond lengths (Å)

C(1)-C(2)	1.533(6)	C(10)-C(23)	1.545(6)	C(1')-O(1')	1.408(3)
C(1)-C(10)	1.545(5)	C(11)-C(12)	1.544(5)	C(2')-C(3')	1.405(4)
C(2)-C(3)	1.544(8)	C(12)-C(13)	1.537(4)	C(3')-C(4')	1.369(4)
C(3)-C(4)	1.543(6)	C(13)-C(14)	1.560(4)	C(4')-C(5')	1.382(4)
C(4)-C(5)	1.545(5)	C(13)-C(18)	1.546(4)	C(4')-O(3')	1.403(3)
C(4)-C(21)	1.536(7)	C(13)-C(25)	1.545(4)	C(5')-C(6')	1.394(4)
C(4)-C(22)	1.518(7)	C(14)-C(15)	1.531(4)	C(5')-O(5')	1.388(4)
C(5)-C(6)	1.544(4)	C(15)-C(16)	1.561(4)	C(6')-Br'	1.884(3)
C(5)-C(10)	1.541(5)	C(16)-C(17)	1.553(4)	C(7')-C(8')	1.497(5)
C(6)-C(7)	1.538(4)	C(17)-C(18)	1.545(4)	C(7')-O(1')	1.375(4)
C(7)-C(8)	1.539(4)	C(17)-C(20)	1.568(4)	C(7')-O(2')	1.170(4)
C(8)-C(9)	1.565(4)	C(17)-C(3')	1.507(4)	C(9')-C(10')	1.483(5)
C(8)-C(14)	1.560(4)	C(18)-C(19)	1.539(4)	C(9')-O(3')	1.374(4)
C(8)-C(24)	1.555(4)	C(19)-C(2')	1.519(4)	C(9')-O(4')	1.185(5)
C(9)-C(10)	1.567(4)	C(1')-C(2')	1.371(4)	C(11')-C(12')	1.513(7)
C(9)-C(11)	1.534(4)	C(1')-C(6')	1.381(5)	C(11')-O(5')	1.336(5)
				C(11')-O(6')	1.171(6)

Bond angles (degrees)

C(2)-C(1)-C(10)	112.7(4)	C(15)-C(16)-C(17)	112.6(2)
C(1)-C(2)-C(3)	110.2(4)	C(16)-C(17)-C(18)	106.8(2)
C(2)-C(3)-C(4)	112.8(4)	C(16)-C(17)-C(20)	112.1(2)
C(3)-C(4)-C(5)	107.4(3)	C(16)-C(17)-C(3')	117.9(2)
C(3)-C(4)-C(21)	105.8(5)	C(18)-C(17)-C(20)	115.5(2)
C(3)-C(4)-C(22)	111.4(4)	C(18)-C(17)-C(3')	100.5(2)
C(5)-C(4)-C(21)	109.7(4)	C(20)-C(17)-C(3')	104.0(2)
C(5)-C(4)-C(22)	115.4(4)	C(13)-C(18)-C(17)	118.7(2)
C(21)-C(4)-C(22)	106.8(4)	C(13)-C(18)-C(19)	120.4(2)
C(4)-C(5)-C(6)	112.4(3)	C(17)-C(18)-C(19)	105.6(2)
C(4)-C(5)-C(10)	118.0(3)	C(18)-C(19)-C(2')	100.6(2)
C(6)-C(5)-C(10)	110.9(3)	C(2')-C(1')-C(6')	120.7(3)
C(5)-C(6)-C(7)	110.6(3)	C(2')-C(1')-O(1')	119.5(3)
C(6)-C(7)-C(8)	113.5(2)	C(6')-C(1')-O(1')	119.8(3)
C(7)-C(8)-C(9)	107.3(2)	C(19)-C(2')-C(1')	129.6(3)
C(7)-C(8)-C(14)	108.2(2)	C(19)-C(2')-C(3')	110.5(3)
C(7)-C(8)-C(24)	107.3(3)	C(1')-C(2')-C(3')	119.9(3)
C(9)-C(8)-C(14)	105.1(2)	C(17)-C(3')-C(2')	110.2(3)
C(9)-C(8)-C(24)	115.6(2)	C(17)-C(3')-C(4')	130.3(3)
C(14)-C(8)-C(24)	112.9(2)	C(2')-C(3')-C(4')	119.3(3)
C(8)-C(9)-C(10)	116.0(3)	C(3')-C(4')-C(5')	120.9(3)
C(8)-C(9)-C(11)	112.1(2)	C(3')-C(4')-O(3')	119.9(3)
C(10)-C(9)-C(11)	115.4(2)	C(5')-C(4')-O(3')	118.9(3)
C(1)-C(10)-C(5)	106.7(3)	C(4')-C(5')-C(6')	119.7(3)
C(1)-C(10)-C(9)	108.8(3)	C(4')-C(5')-O(5')	120.1(3)
C(1)-C(10)-C(23)	108.0(3)	C(6')-C(5')-O(5')	120.0(3)
C(5)-C(10)-C(9)	106.7(3)	C(1')-C(6')-C(5')	119.5(3)
C(5)-C(10)-C(23)	115.3(3)	C(1')-C(6')-Br'	121.1(2)
C(9)-C(10)-C(23)	111.1(3)	C(5')-C(6')-Br'	119.4(2)
C(9)-C(11)-C(12)	112.1(3)	C(8')-C(7')-O(1')	109.4(3)
C(11)-C(12)-C(13)	111.7(3)	C(8')-C(7')-O(2')	127.9(4)
C(12)-C(13)-C(14)	106.9(2)	O(1')-C(7')-O(2')	122.7(3)
C(12)-C(13)-C(18)	114.4(3)	C(10')-C(9')-O(3')	109.6(4)
C(12)-C(13)-C(25)	109.3(3)	C(10')-C(9')-O(4')	128.3(4)
C(14)-C(13)-C(18)	106.0(2)	O(3')-C(9')-O(4')	122.1(4)
C(14)-C(13)-C(25)	116.0(3)	C(12')-C(11')-O(5')	108.4(5)
C(18)-C(13)-C(25)	104.4(2)	C(12')-C(11')-O(6')	128.1(5)
C(8)-C(14)-C(13)	115.4(2)	O(5')-C(11')-O(6')	123.5(5)
C(8)-C(14)-C(15)	118.0(2)	C(1')-O(1')-C(7')	115.9(2)
C(13)-C(14)-C(15)	110.3(2)	C(4')-O(3')-C(9')	117.9(3)
C(14)-C(15)-C(16)	110.6(2)	C(5')-O(5')-C(11')	118.8(3)

Selected torsion angles (degrees)

C(1')-O(1')-C(7')-O(2')	-0.8(6)	C(9')-O(3')-C(4')-C(3')	-105.9(6)
C(7')-O(1')-C(1')-C(2')	85.5(5)	C(5')-O(5')-C(11')-O(6')	-0.9(8)
C(4')-O(3')-C(9')-O(4')	3.0(6)	C(11')-O(5')-C(5')-C(4')	85.3(7)

partially releases the intramolecular steric strain. The 1-5 shortest intramolecular methyl-methyl contacts are: 3.086 Å [C(24)-C(25)], 3.132 Å [C(23)-C(24)] and 3.249 Å [C(22)-C(23)].

In Table 4 are reported for each ring the conformational parameters according to Cremer and Pople[7]. The A, B and C rings exhibit conformations close to that of ideal chair, while ring D adopts a twist boat conformation. The pentacyclic ring E has an envelope conformation with C(18) 0.544 Å out of the best plane through C(17), C(19), C(2') and C(3') atoms: the torsion angle about the double bond C(17)-C(3')-C(2')-C(19) is 3.5°.

The aromatic ring is planar within the esd and the four ring substituents Br', O(1'), O(3') and O(5') deviate from this plane 0.067, 0.045, 0.162 and 0.123 Å, respectively. The three acetyl groups make with the aromatic best plane dihedral angles of 85°, 101° and 97°.

A stereoview of the arrangement of the molecules in the unit cell is shown in Fig. 2. In the molecule are not present H-bond donors and the packing is governed only by Van der Waals interactions.

It seems very attractive in view of the established stereochemistry of Disideins and Toxistylides to suppose a common biosynthetic origin for all these sesterterpenoids. The carbonium ion (*11*) could be a key-intermediate which should, by a direct cyclization involving C-3' and C-17, lead to the carbon skeleton of Disidein or, by an unprecedented concerted series of methyl and hydrogen shifts, generate Toxistylide-A and -B.

TABLE 4 - Puckering parameters for compound *7*

Ring	Sequence	θ(°)	Q(Å)	q_2(Å)	q_3(Å)	ϕ_2(°)
A	C(1)......C(10)	3.79	0.5700	0.0377	0.5688	40.27
B	C(5)......C(10)	4.56	0.5883	0.0468	0.5864	356.57
C	C(14).....C(8)	2.78	0.5978	0.0290	0.5971	338.55
D	C(14).....C(13)	87.21	0.7867	0.7858	0.0383	22.95
E	C(18).....C(19)	-	0.3533	0.3533	-	184.80

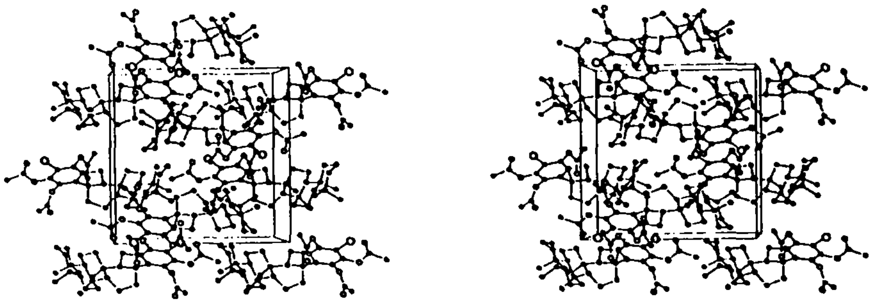

Fig. 2 - Stereoview of the molecular packing of *7*.
H-atoms have been omitted.

EXPERIMENTAL

NMR spectra were recorded on Bruker WM 500 and WM 250 spectrometers (δppm/TMS). The 2D NMR spectra were obtained using Bruker's microprograms. Mass spectra were taken on AEI MS-30 and Kratos MS-50 instruments. IR spectra were recorded on a Perkin-Elmer 257 spectrophotometer. UV spectra were obtained on a Shimadzu Baush & Lomb Spectronic 210 Spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Silica gel chromatography was performed using, pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder. Preparative HPLC purifications were carried out on a Waters apparatus equipped with μ-Porasil column and with an UV detector (260 nm).

Isolation procedure. *D. pallescens* was collected in Naples Bay and extracted as previously described[3]. The n-butanol soluble material (16 g) was treated with Ac_2O (20 ml) in pyridine (2 ml) at reflux for 2 h. The excess of reagents was removed *in vacuo* and the residue was chromatographed on a SiO_2 column (benzene-diethyl ether, 95:5) yielding two main fractions, single spots on TLC (Rf 0.4 and 0.3; light petrol--Et_2O, 7:3). The less polar fraction (200 mg) was subjected to HPLC [μ-Porasil, 3.9 mm (ID) x 30 cm; n-hexane-ethyl acetate, 97:3; flow rate 3.5 ml/min] yielding *10* (3 mg, Rt 8 min), *6* (80 mg, Rt 17 min), *7* (20 mg, Rt 18 min). The second fraction (8 g) was crystallized from MeOH giving 6 g of pure *5*.

6' Cl-Disidein, *6*: $C_{37}H_{51}O_6Cl$, determined by HREIMS on M^+, obsd. 626.3379, calcd. 626.3374; mp 158-159°; $[\alpha]_D$ + 22.1 (c 2, $CHCl_3$); UV λmax (MeOH) 265 (ε 590) and 276 nm (s, ε 510); IR (liquid film); 2935, 2753, 2259, 1782, 1445, 1370, 1186, 1170, 1100 cm^{-1}; EIMS, m/z (%): 628 (M^++2, 0.1), 626 (M^+, 0.3), 612 (0.6), 610 (1.8), 586 (6), 584 (18), 544 (12), 542 (37), 502 (30); 500 (100), 466 (7.5), 225 (34), 205 (10), 191 (20). ^{1}H-NMR ($CDCl_3$) δ: 2.57 (H-19, 1H, dd J=6.6 and 14.8 Hz), 2.48 (H-19, 1H, dd, J=13 and 14.8 Hz), 2.34 (3H, s), 2.284 (3H, s), 2.277 (3H, s), 2.08 (H-16, dd, J=9 and 13 Hz), 1.98 (H-18, 1H, dd, J=6.6 and 13 Hz), 1.29 (3H, s), 0.99 (3H, s), 0.85 (3H, s), 0.84 (3H, s), 0.83 (3H, s), 0.81 (3H, s). The remaining signals resonate between 1.83 and 0.78 δ. ^{13}C-NMR ($CDCl_3$) δ: 167.8, 167.2, 166.7, 146.3, 141.4, 138.8, 135.9, 134.7, 118.5, 61.9, 57.3, 56.7, 51.8, 46.2, 42.0, 41.9, 39.5, 38.0, 37.5, 36.5, 35.9, 33.7, 33.1, 25.7, 24.8, 23.6, 21.0, 20.1, 19.9, 18.4, 18.2, 18.0, 17.9, 16.5, 15.7.

6' Br-Disidein, *7*: $C_{31}H_{51}O_6Br$, determined by HREIMS on M^+, obsd. 670.2875, calcd. 670.2869; mp 159-160°; $[\alpha]_D$ + 24.2 (c 2.5, $CHCl_3$); UV λmax (MeOH) 267 (ε 629) and 276 nm (s, ε 560); IR (liquid film) 2935, 1853, 1780, 1439, 1370, 1186, 1167, 1100, 1020 cm^{-1}. EIMS, m/z (%); 672 (M^++2, 0.32), 670 (M^+, 0.35), 658 (1.5), 656 (1.6), 630 (14), 628 (14.3), 588 (36), 586 (36.5), 546 (99), 544 (100), 508 (6.3), 466 (7.9), 271 (17), 269 (17.5), 205 (2). ^{1}H-NMR ($CDCl_3$): resonances almost identical to those of *6*. The acetyl protons resonate at δ 2.34, 2.29 and 2.27. ^{13}C-NMR ($CDCl_3$) δ : 167.8, 167.1, 166.7, 147.3, 142.6, 140.1, 136.0, 135.0, 108.8, 61.9, 57.4, 56.7, 51.8, 46.3, 42.0, 41.9, 39.5, 38.0, 37.5, 36.5, 35.9, 33.6, 33.1, 25.9, 24.7, 23.5, 21.0, 20.3, 20.1, 18.4, 18.2, 18.0, 17.9, 16.4, 15.7.

Triacetyl Disidein, *5*: ^{1}H- and ^{13}C-NMR: Table 1.
Other spectroscopic data have been already reported[3].

Diacetyl 5' dehydroxy Disidein, *10*: MS, m/z (%): 534 (M^+, 6) 492 (30), 450 (100), 259 (6), 205 (10), 191 (14). ^{1}H-NMR ($CDCl_3$). All the resonances were almost identical to those observed in the spectra of *5*, *6* and *7* except the presence of an AB system (2H) at δ 6.82 and of only two acetyl methyls at δ 2.30 and 2.27.

Synthesis of *6*: 800 mg of *5* were dissolved in CCl_4 and treated with bubbling chlorine for 3 h. After removal of the solvent, the mixture of reaction was purified on silica-gel column (light petrol-Et_2O; 7:3) giving a product (110 mg) identical to *6*.

Synthesis of *7*: 330 mg of *5* were dissolved in acetic acid (2 ml) and treated with bromine (300 mg) for 36 h. After concentration, the residue was purified by silica-gel column yielding a product (50 mg) identical to *7*.

Crystal data and structure determination. Single crystals of *6* and *7* derivatives were obtained by careful recrystallization from methanol. They are strictly isomorphous and belong to the orthorhombic system, space group $P2_12_12_1$ with Z = 4 and cell parameters a = 13.779(2), b = 14.332(2), c = 17.844(2) Å, V = 3524(1) Å^3, M = 671.7, D_C = 1.266 g/cm^3 for $C_{37}H_{51}O_6Br$ and a = 13.800(5), b = 14.310(5), c = 17.692(7) Å, V = 3494(4) Å^3, M = 627.3 D_C = 1.193 g/cm^3 for $C_{37}H_{51}O_6Cl$. The crystals of bromocompound showed a better diffraction pattern and were used for a detailed structural analysis. Intensities of 4039 independent reflections with θ ≤ 75° were collected at room temperature, using a single crystal (0.67x0.55x0.47 mm) mounted on an Enraf-Nonius CAD-4 diffractometer on line with a PDP11/34 computer, with ω-2θ scan mode and Ni-filtered CuKα radiation (λ = 1.54178 Å). The equipment and crystal stability were checked by three standard reflections monitored at intervals of 5h (≤ 4% variation) during all the data collection. 3612 intensities with I ≥ 3σ(I), with σ based on counting statistics, were considered observed. Intensities were corrected for Lorentz and polarization factors but not for absorption effect (μ = 19.2 cm^{-1}). The structure was solved by Patterson method and difference Fourier syntheses. The refinement of the positional and anisotropic temperature parameters for non hydrogen atoms was carried out by full-matrix (on F) least-squares cycles and provided the discrepancy index R = 0.041 for the 3612 observed reflections and 397 variables; R_w = 0.064 with w = $1/\sigma^2(F_O)$. The hydrogen atoms were generated at the expected positions (C-H distance = 1.08 Å), taking into account, for methyl groups, the indications of the difference Fourier map. All

the hydrogens were included in the last refinement as fixed atoms and with the isotropic thermal parameters set equal to $B_{eq.}$ of the parent atoms. The highest peak in the final difference Fourier synthesis showed a density of 0.3 eÅ^{-3}.
The absolute configuration was established selecting 120 reflections for which the effects of anomalous intensities were calculated for all the Friedel's pairs. Furthermore the enantiomorphous molecule refined to a significantly higher R value (0.049). Diffraction data for the Cl-derivative *6* were also measured (θ_{max} = 65°)and processed using the same procedures described for *7*. In this case however only 1340 independent reflections had $I \geqslant 3\sigma(I)$. The model of *7* provided a starting set of coordinates, which were refined isotropically. Only Cl and O atoms were treated anisotropically. The final discrepancy index was R = 0.079 (R_w = 0.106). Bond lengths and angles correlate well with those found for the bromoderivative (see discussion), although their estimated errors are much higher. For these reasons the structure of *6* is not further discussed.
For this crystallographic work the equipment of the "Centro di Metodologie Chimico-Fisiche dell'Università di Napoli" and the SDP package was used. Scattering factors were taken from Cromer and Waber[8].
The final values of the positional and equivalent thermal parameters for non-hydrogen atoms of the bromocompound are given in Table 2 with the atomic numbering reported in Fig. 1. Bond distances and bond angles for the same structure are listed in Table 3 together with some selected torsion angles. The anisotropic parameters, H-parameters and structure factors for the bromocompound, together with the thermal and positional parameters and structure factors for the chlorocompound have been deposited.

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