Secondary Mould Metabolites. Part 19.[1] Structure Elucidation and Absolute Configuration of Melledonals B and C, Novel Antibacterial Sesquiterpenoids from *Armillaria mellea*. *X*-Ray Molecular Structure of Melledonal C

Alberto Arnone, Rosanna Cardillo, and Gianluca Nasini
Dipartimento di Chimica del Politecnico, Centro del C.N.R. per le Sostanze Organiche Naturali, Politecnico di Milano, Piazza L. da Vinci 32, I 20133 Milano, Italy
Stefano Valdo Meille
Dipartimento di Chimica del Politecnico di Milano, Piazza L. da Vinci 32, I 20133 Milano, Italy

The structure, absolute configuration, and preferred conformation, both in the solid state and in solution, of melledonal C (**11**), a $\Delta^{2,3}$-protoilludene sesquiterpenoid from *Armillaria mellea*, have been established by a combination of *X*-ray and n.m.r. procedures. Ring A of the protoilludene skeleton was found in the crystal in a slightly twisted envelope conformation with C(10) displaced 0.55 Å below the C(9), C(13), C(12), C(11) plane; ring B is a (7β,9α) flattened half-chair and ring C assumes a (4β,6β) highly puckered conformation. A strong intramolecular hydrogen bond between the 4-OH proton and the oxygen atom of 10-OH was evidenced in the solid state as well as in solution ([$^2H_6$]acetone) using the SIMPLE ^{1}H n.m.r. method (secondary isotope multiplet n.m.r. of partially labelled entities). Contrary to what is observed in solution, in the solid state there is no hydrogen bonding between the 3'-OH proton and the oxygen atom of the adjacent CO_2R group. The structure of melledonals A (**9**) and B (**10**), and the absolute configuration at C(13) of melleolides C (**6**) and D (**7**) have also been elucidated by n.m.r. and chemical evidence.

In earlier papers we have reported on the isolation and structural characterization of new biogenetically related compounds with a protoilludene skeleton, produced by various strains of Basidiomycetes, *viz. Laurilia sulcata*,[1] *Armillaria mellea*,[2] and *Clitocybe* spp.[3,4]

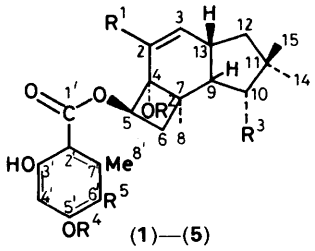
Previous work on the metabolites isolated from *A. mellea* concerned a number of sesquiterpenoids containing the $\Delta^{2,3}$-protoilludene-4,5-diol skeleton, such as melleolide (**1**),[5] 4-*O*-methyl melleolide (**2**),[6] armillarin (**3**), and armillaridin (**4**);[7] the $\Delta^{2,3}$-protoilludene-4,5,10-triol skeleton, such as melleolide B (**5**);[2] and the $\Delta^{2,3}$-protoilludene-4,5,10,13-tetraol skeleton, such as melleolides C (**6**) and D (**7**),[2] melledonol (**8**), and melledonal (**9**).[8]

Further investigations on a pure strain of *A. mellea* grown on glucose–malt–peptone–glycerine–agar medium led to the isolation of two new $\Delta^{2,3}$-protoilludene-4,5,10,13-tetraol sesquiterpenoids: melledonals B (**10**) and C (**11**), and the known melledonal A (**9**). The crystalline metabolites, which exhibited marked antibacterial activity, were isolated by extraction of the mycelium of the fungus with ethyl acetate followed by flash chromatography on silica gel.

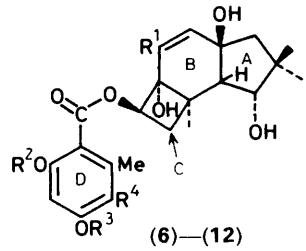
It should be noted that the structure of melledonal A (**9**) * was found to be identical with that reported by Donnelly *et al.* in a preliminary note, while some physical properties (m.p., $[\alpha]_D$) and the preferred conformation in solution appear to be significantly different.

In this paper we report on the structural elucidation of melledonals A—C (**9**)—(**11**) using *X*-ray and n.m.r. techniques, and on the assignment of the absolute configuration at C(13) of the previously isolated melleolides C (**6**) and D (**7**).[2] The preferred conformation of melledonal C (**11**) in the solid state and in [$^2H_6$]acetone is also discussed.

Melledonal C (**11**) crystallized from EtOAc–hexane, m.p. 200—205 °C, and analysed for $C_{24}H_{29}ClO_8$ (M^+, 480/482); it had $[\alpha]_D$ +121° (*c* 0.1 in MeOH); $\lambda_{max.}$(EtOH) 214, 257, and 304 nm (ε 29 300, 9 300, and 4 000, respectively); $\nu_{max.}$(KBr) 1 690 (unsaturated aldehyde) and 1 650 cm^{-1} (chelated ester). Its structure is based on a single-crystal *X*-ray crystallographic analysis and on ^{1}H and ^{13}C n.m.r. studies.



(1)—(5)



(6)—(12)

(**1**) R^1 = CHO, R^2 = R^3 = R^4 = R^5 = H

(**2**) R^1 = CHO, R^2 = Me, R^3 = R^4 = R^5 = H

(**3**) R^1 = CHO, R^2 = R^3 = R^5 = H, R^4 = $^{9'}$Me

(**4**) R^1 = CHO, R^2 = R^3 = H, R^4 = Me, R^5 = Cl

(**5**) R^1 = CH_2OH, R^2 = R^5 = H, R^3 = OH, R^4 = Me

(**6**) R^1 = CH_2OH, R^2 = R^4 = H, R^3 = Me

(**7**) R^1 = CH_2OH, R^2 = H, R^3 = Me, R^4 = Cl

(**8**) R^1 = CH_2OH, R^2 = R^3 = R^4 = H

(**9**) R^1 = CHO, R^2 = R^3 = R^4 = H

(**10**) R^1 = CHO, R^2 = R^3 = H, R^4 = Cl

(**11**) R^1 = CHO, R^2 = H, R^3 = $^{9'}$Me, R^4 = Cl

(**12**) R^1 = CHO, R^2 = R^3 = Ac, R^4 = H

X-Ray Analysis of Melledonal C (**11**).—An ORTEP view of melledonal C (**11**), in the correct absolute configuration,

* Melledonal refers to the metabolite isolated by Donnelly *et al.*[8] and melledonal A (**9**) to the corresponding metabolite isolated by us.

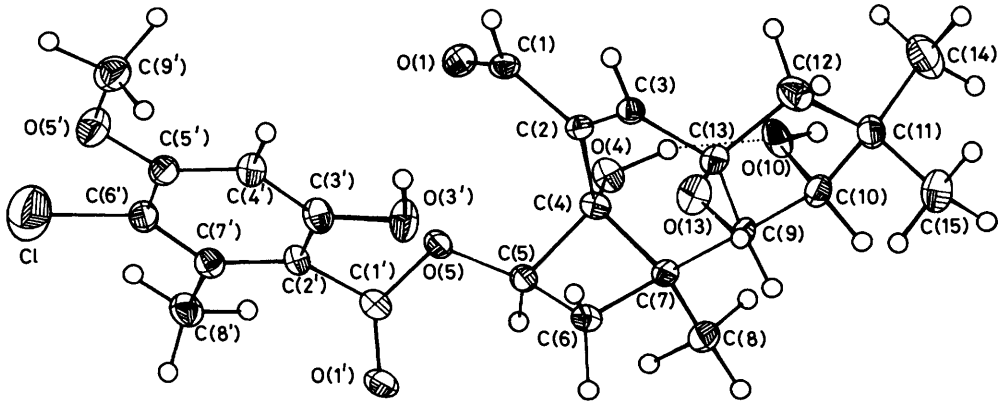


Figure 1. An ORTEP drawing of melledonal C (**11**)

Table 1. Atomic co-ordinates for compound (**11**) with e.s.d.s in parenthesis (crystallographic numbering)

Atom	*x*	*y*	*z*
Cl	15 318(6)	51 075(7)	45 944(23)
C(1')	616(2)	2 294(2)	1 521(5)
C(2')	934(2)	2 756(2)	3 095(5)
C(3')	1 130(2)	2 257(2)	4 599(6)
C(4')	1 458(2)	2 630(3)	6 095(6)
C(5')	1 575(2)	3 508(3)	6 073(6)
C(6')	1 370(2)	4 002(2)	4 572(6)
C(7')	1 044(2)	3 651(3)	3 060(5)
C(8')	841(2)	4 191(3)	1 419(7)
C(9')	2 147(2)	3 432(3)	8 943(7)
O(1')	85(1)	2 435(2)	962(4)
O(3')	980(2)	1 404(2)	4 579(4)
O(5')	1 884(1)	3 935(2)	7 453(5)
C(1)	2 148(2)	751(2)	858(6)
C(2)	1 633(2)	143(2)	513(5)
C(3)	1 617(2)	−562(2)	1 561(5)
C(4)	1 155(2)	336(2)	−1 000(5)
C(5)	702(2)	1 060(2)	−400(5)
C(6)	284(2)	365(2)	484(6)
C(7)	574(2)	−293(2)	−906(5)
C(8)	186(2)	−306(3)	−2 701(6)
C(9)	717(2)	−1 222(2)	−285(5)
C(10)	996(2)	−1 807(3)	−1 779(6)
C(11)	1 281(2)	−2 581(3)	−728(7)
C(12)	1 540(2)	−2 157(3)	1 057(7)
C(13)	1 168(2)	−1 305(2)	1 411(5)
C(14)	1 792(3)	−3 054(3)	−1 804(10)
C(15)	746(3)	−3 223(3)	−246(9)
O(1)	2 306(1)	1 354(2)	−100(4)
O(4)	1 455(1)	430(2)	−2 770(3)
O(5)	982(1)	1 688(2)	825(4)
O(10)	1 481(1)	−1 317(2)	−2 729(4)
O(13)	852(1)	−1 320(2)	3 184(4)

(4*S*,5*R*,7*R*,9*S*,10*R*,13*R*), and with the appropriate numbering scheme, is shown in Figure 1; final atomic co-ordinates are given in Table 1 while bond lengths and angles are listed in Tables 2 and 3, respectively. Some relevant torsion angles are reported in Table 4. The configurations at the chiral centres C(5), C(7), and C(9) are the same as those proposed for the orsellinate* of armillol, a related metabolite containing the $\Delta^{2,4}$-protoilludene-3,5-diol skeleton, based on *X*-ray and circular dichroism measurements.[9,10]

* 4,6-Dihydroxy-*o*-toluate.

Table 2. Bond lengths (Å) for compound (**11**), with e.s.d.s in parenthesis

Cl–C(6')	1.741(4)	C(3)–C(13)	1.497(5)
C(1')–C(2')	1.494(5)	C(4)–C(5)	1.538(5)
C(1')–O(1')	1.217(5)	C(4)–C(7)	1.573(5)
C(1')–O(5)	1.315(4)	C(4)–O(4)	1.425(4)
C(2')–C(3')	1.387(5)	C(5)–C(6)	1.530(5)
C(2')–C(7')	1.402(5)	C(5)–O(5)	1.437(4)
C(3')–C(4')	1.401(6)	C(6)–C(7)	1.549(5)
C(3')–O(3')	1.354(5)	C(7)–C(8)	1.526(6)
C(4')–C(5')	1.380(6)	C(7)–C(9)	1.534(5)
C(5')–C(6')	1.387(6)	C(9)–C(10)	1.519(5)
C(5')–O(5')	1.356(5)	C(9)–C(13)	1.550(5)
C(6')–C(7')	1.394(6)	C(10)–C(11)	1.536(6)
C(7')–C(8')	1.504(6)	C(10)–O(10)	1.448(5)
C(9')–O(5')	1.432(6)	C(11)–C(12)	1.537(7)
C(1)–C(2)	1.464(5)	C(11)–C(14)	1.519(7)
C(1)–O(1)	1.204(5)	C(11)–C(15)	1.548(7)
C(2)–C(3)	1.322(5)	C(12)–C(13)	1.556(6)
C(2)–C(4)	1.515(5)	C(13)–O(13)	1.435(5)

The cyclobutane ring is highly puckered (φ 34.8°) and the average values of bond lengths *d*(Å) (1.548), bond angles θ(°) (87.3), and ring torsion angles ω(°) (24.6) are in agreement with literature values[11] for cyclobutane rings with puckering angles φ between 30 and 40°.

The cyclohexene ring is distorted from the C_2 symmetry of the half-chair conformation found in the gas phase[12] and is flattened because of condensation with the cyclobutane systems.

The conformation of the cyclopentane ring may be described as a slightly twisted envelope;[13] C(10) lies 0.55 Å below the least-squares plane through C(9), C(13), C(12), and C(11), and the angle between this plane and the C(9), C(10), C(11), plane is 36.4°.

As a result of this conformation of the protoilludene skeleton, it is possible to form a strong intramolecular hydrogen bond [O(10)···H(04) 1.93 Å; O(4)–H(4) 0.97 Å, O(4)···O(10) 2.699 Å, O(4)–H(04)···O(10) 135°]. Moreover this conformation agrees with the one determined for the melleolides C and D,[2] while the cyclopentane ring conformation is different for compounds (**1**)[5] and (**2**)[6] which, however, do not carry hydroxy groups either at C-10 or at C-13.

A distinctive feature of the solid-state conformation of melledonal C (**11**) is the absence of the intramolecular hydrogen bonding between the ester carbonyl oxygen O(1') and the *ortho*-hydroxy hydrogen H(03'). This bonding is apparent (*vide infra*) in solution and has also been observed in the crystal structures of similar molecules. However, it is prohibited in the crystalline

Table 3. Bond angles (°) for compound (**11**), with e.s.d.s in parentheses

Cl-C(6')-C(5')	117.9(2)	C(3)-C(13)-C(12)	109.6(3)
Cl-C(6')-C(7')	119.1(2)	C(3)-C(13)-O(13)	104.3(3)
C(1')-C(2')-C(3')	117.2(2)	C(4)-C(5)-C(6)	88.2(3)
C(1')-C(2')-C(7')	122.2(2)	C(4)-C(5)-O(5)	113.6(2)
C(1')-O(5)-C(5)	117.6(2)	C(4)-C(7)-C(6)	86.3(2)
C(2')-C(1')-O(1')	125.6(2)	C(4)-C(7)-C(8)	113.4(2)
C(2')-C(1')-O(5)	110.8(3)	C(4)-C(7)-C(9)	115.8(2)
C(2')-C(3')-C(4')	120.9(3)	C(5)-C(4)-C(7)	86.8(2)
C(2')-C(3')-O(3')	117.4(3)	C(5)-C(4)-O(4)	117.0(2)
C(2')-C(7')-C(6')	117.0(3)	C(5)-C(6)-C(7)	88.0(3)
C(2')-C(7')-C(8')	120.9(2)	C(6)-C(5)-O(5)	117.6(2)
C(3')-C(2')-C(7')	120.7(2)	C(6)-C(7)-C(8)	109.5(3)
C(3')-C(4')-C(5')	119.0(3)	C(6)-C(7)-C(9)	120.4(2)
C(4')-C(3')-O(3')	121.7(2)	C(7)-C(4)-O(4)	116.9(2)
C(4')-C(5')-C(6')	119.5(3)	C(7)-C(9)-C(10)	115.4(2)
C(4')-C(5')-O(5')	123.8(3)	C(7)-C(9)-C(13)	115.2(2)
C(5')-C(6')-C(7')	122.9(2)	C(8)-C(7)-C(9)	109.8(3)
C(5')-O(5')-C(9')	117.9(3)	C(9)-C(10)-C(11)	105.7(3)
C(6')-C(5')-O(5')	116.7(3)	C(9)-C(10)-O(10)	107.4(3)
C(6')-C(7')-C(8')	122.1(2)	C(9)-C(13)-C(12)	104.9(3)
O(1')-C(1')-O(5)	123.6(2)	C(9)-C(13)-O(13)	113.7(2)
O(1')-C(1')-O(5)	123.6(2)	C(10)-C(9)-C(13)	105.1(3)
C(1)-C(2)-C(3)	116.9(2)	C(10)-C(11)-C(12)	102.5(3)
C(1)-C(2)-C(4)	119.8(2)	C(10)-C(11)-C(14)	114.1(3)
C(2)-C(1)-O(1)	127.4(2)	C(10)-C(11)-C(15)	108.5(3)
C(2)-C(3)-C(13)	127.4(2)	C(11)-C(10)-O(10)	110.8(3)
C(2)-C(4)-C(5)	111.4(2)	C(11)-C(12)-C(13)	108.2(3)
C(2)-C(4)-C(7)	112.0(2)	C(12)-C(11)-C(14)	111.7(3)
C(2)-C(4)-O(4)	110.7(2)	C(12)-C(11)-C(15)	110.6(4)
C(3)-C(2)-C(4)	123.3(2)	C(12)-C(13)-O(13)	111.6(3)
C(3)-C(13)-C(9)	112.8(2)	C(14)-C(11)-C(15)	109.3(3)

melledonal C by the mutual orientation of O(1') and O(3') (see Table 4 and Figure 1), and by the consequently excessive distance[14] between the two oxygen atoms [O(1')···O(3') 3.588(6) Å]. This arrangement is likely to be determined by the overall packing in the crystal; in fact O(1'), O(3'), and all the oxygen atoms of melledonal C with the exception of O(5) and O(5') participate in three-dimensional network of intra- and inter-molecular hydrogen bonds (see Table 5).

^{1}H And ^{13}C N.m.r. Analysis of Melledonal C (**11**).—Melledonal C (**11**) had spectral characteristics in accord with the proposed structure; the ^{1}H and ^{13}C n.m.r. resonances have been completely assigned and are collected in Tables 6 and 7. Several structural features are evident from the ^{1}H n.m.r. spectrum in [$^2H_6$]acetone, such as the presence of an aldehydic

Table 4. Selected torsion angles (°) of compound (**11**), with e.s.d.s in the range 0.4—0.8°

C(2')-C(1')-O(5)-C(5)	−166.3	C(4)-C(7)-C(9)-C(13)	−43.9
C(3')-C(2')-C(1')-O(1')	−120.6	C(5)-C(4)-C(7)-C(6)	24.2
C(7')-C(2')-C(1')-O(1')	60.6	C(6)-C(5)-C(4)-C(7)	−24.4
O(1')-C(1')-O(5)-C(5)	12.2	C(8)-C(7)-C(4)-O(4)	33.4
C(2)-C(3)-C(13)-C(9)	−4.7	C(8)-C(7)-C(9)-C(10)	−51.1
C(2)-C(4)-C(7)-C(9)	34.5	C(9)-C(10)-C(11)-C(12)	36.4
C(3)-C(2)-C(1)-O(1)	−167.8	C(9)-C(10)-C(11)-C(14)	157.3
C(3)-C(2)-C(4)-C(7)	−11.3	C(9)-C(10)-C(11)-C(15)	−80.6
C(3)-C(13)-C(9)-C(7)	28.1	C(9)-C(13)-C(12)-C(11)	3.4
C(4)-C(2)-C(3)-C(13)	−3.6	C(10)-C(9)-C(13)-C(12)	19.2
C(4)-C(5)-C(6)-C(7)	24.8	C(10)-C(11)-C(12)-C(13)	−24.1
C(4)-C(7)-C(6)-C(5)	−24.3	C(11)-C(10)-C(9)-C(13)	−35.1

Table 5. Hydrogen bonding in melledonal C (**11**)

A···H—B	A···B (Å)	A···H (Å)	A···$\widehat{H}$—B (°)	Symmetry operation for B
O(10)···H(04)—O(4)	2.699(6)	1.93	135	x, y, z
O(4)···H(03')—O(3')	2.625(7)	1.54	152	$x, y, z-1$
O(1')···H(013)—O(13)	2.843(7)	1.78	169	$-x, \frac{1}{2}+y, \frac{1}{2}-z$
O(13)···H(010)—O(10)	3.217(7)	2.46	129	$x, y, z+1$
O(1')···H(010)—O(10)	3.099(7)	2.57	112	$\frac{1}{2}-x, -y, \frac{1}{2}+z$

Table 6. ^{1}H N.m.r. chemical shifts (δ_H)[a] and ^{1}H-^{1}H coupling constants (J/Hz) for compounds (**9**)—(**12**) in [$^2H_6$]acetone

Proton	(9)	(10)	(11)	(12)	J	(9)	(10)	(11)	(12)
1	9.59 (9.51)[b]	9.60	9.59 (9.51)	9.56 (9.44)	3,9	1.2	1.2	1.2	1.2
3	6.97 (6.86)	6.96	6.96 (6.85)	6.85 (6.74)	5,6α	8.4	8.3	8.3	8.3
5	5.77 (5.74)	5.71	5.72 (5.72)	5.51 (5.54)	5,6β	8.9	8.8	8.9	8.7
6α	1.99 (2.08)	2.02	2.02 (2.09)	2.05 (2.11)	6α,6β	11.1	11.1	11.1	11.1
6β	2.29 (2.08)	2.28	2.28 (2.09)	2.13 (1.94)	6β,8	0.7	0.7	0.7	0.7
8	1.41 (1.42)	1.41	1.41 (1.42)	1.40 (1.39)	9,10	3.9	3.9	3.9	3.9
9	2.55 (2.54)	2.55	2.55 (2.54)	2.53 (2.51)	9,10-OH	0.7	0.7	0.7	0.7
10	3.74 (3.76)	3.75	3.74 (3.75)	3.74 (3.72)	10,12β	1.1	1.1	1.1	1.1
12α	2.02 (2.15)	2.02	2.01 (2.15)	1.98 (2.10)	10,10-OH	2.5	2.6	2.6	2.6
12β	1.95 (1.89)	1.95	1.94 (1.88)	1.91 (1.86)	12α,12β	14.0	13.9	14.0	14.0
14	0.98 (1.03)	0.98	0.98 (1.03)	0.97 (1.01)	12α,15	0.6	0.6	0.6	0.6
15	1.17 (1.19)	1.17	1.17 (1.19)	1.16 (1.17)	4',6'	2.6[e]			2.2
4'	6.22 (6.24)	6.45	6.51 (6.41)	6.84 (6.74)	4',8'	0.5[e]	0.5	0.5	0.5
6'	6.22 (6.14)			6.94 (6.85)	6',8'	0.9[e]			0.8
8'	2.29 (2.27)	2.42	2.41 (2.43)	2.36 (2.36)					
4-OH	4.30 *c*	4.27	4.26 *c*	4.27 *c*					
10-OH	3.40 *c*	3.44	3.46 *c*	3.49 *c*					
13-OH	4.57 *c*	4.57	4.58 *c*	4.50 *c*					
3'-OR	11.62 (11.55)	10.91	11.08 (11.28)	2.26[d] (2.27)[d]					
5'-OR	9.23 (6.17)	9.40	3.92 (3.88)	2.21[d] (2.22)[d]					

[a] Chemical shifts refer to the protio form. [b] Values in parentheses are chemical shifts in $CDCl_3$. [c] Not assigned. [d] Assignments may be interchanged. [e] Coupling constants observed in $CDCl_3$.

Table 7. ^{13}C N.m.r. chemical shifts (δ_C) and ^1H–^{13}C coupling constants (J/Hz) for compounds (**9**)—(**11**) in [$^2H_6$]acetone

	(9)			(10)			(11)		
Carbon	δ_C[a]	^1J	$>^1J$	δ_C[a]	^1J	$>^1J$	δ_C[a]	^1J	$>^1J$
1	195.69 Dd	177	8	195.72 Dd	177	8	195.70 Dd	177.5	8
2	135.03 Sbr dd		23.5, 3	135.03 Sbr dd		23.5, 3	135.04 Sbr dd		23.5, 3
3	151.32 Dm	158		151.09 Dm	159		151.30 Dm	159	
4	75.54[b] Sm			75.34[b] Sm			75.33[b] Sm		
5	74.71 Ddd	159	8, 2.5	75.39 Dm	159		75.40 Dm	159	
6	32.79 Tm	139		33.10 Tm	139		33.03 Tm	139	
7	36.83 Sm			37.12 Sm			37.10 Sm		
8	21.40 Qm	126		21.44 Qm	126		21.45 Qm	126	
9	55.68 Dm	126		55.71 Dm	125		55.69 Dm	125	
10	82.30 Dm	148		82.41 Dm	149		82.39 Dm	149	
11	41.82 Sm			41.88 Sm			41.88 Sm		
12	55.31 Tm	131		55.41 Tm	130		55.38 Tm	130	
13	76.93[b] Sm			76.98[b] Sm			76.97[b] Sm		
14	23.97 Qm	125		24.00 Qm	125		23.40 Qm	125	
15	28.51 Qm	125		28.56 Qm	125		28.55 Qm	125	
1′	171.53 Sbr d		3	170.40 Sbr d		3	170.45 Sbr d		3
2′	105.34 Sm			108.42 Sm			108.35 Sm		
3′	166.48 Sm			162.49 Sdd		4,4	163.02 Sdd		4.5, 4.5
4′	101.80 Ddd	161	7, 4.5	102.78 Dd	163	7	99.58 Dd	163.5	7.5
5′	163.30 Sdd		3, 3	158.55 Sd		4	160.38 Sm		
6′	112.32 Ddq	161	5.5, 5.5	114.93 Sm			115.87 Sm		
7′	144.18 Sq		6	140.10 Sq		6	139.63 Sq		6
8′	24.44 Qd	129.5	6.5	19.77 Qs	129.5		19.66 Qs	129.5	
9′							56.89 Qs	146	

[a] Relative to internal Me_4Si. Capital letters refer to the pattern resulting from directly bonded (C,H) couplings [^1J(CH)] and small letters to that from (C,H) couplings over more than one bond [$^{>1}J$CH)]. S = Singlet, D or d = doublet, T = triplet, Q or q = quartet, m = multiplet, and br = broad (no fine structure but the line is noticeably broadened, indicating unresolved coupling. [b] Assignments within each column may be interchanged.

(1-H; δ_H 9.59), a vinylic (3-H; δ_H 6.96), and an aromatic (4′-H; δ_H 6.51) proton; of an OMe group (9′-H_3; δ_H 3.92); and of four broad methyl group signals (8-, 14-, 15-, and 8′-H_3; δ_H 1.41, 0.98, 1.17, and 2.41, respectively). The AB spin system (^2J 14.0 Hz) resonating at δ_H 2.01 and 1.94 was assigned to 12-H_2. The A (12-H_α) and B (12-H_β) portions showed also W-type long-range couplings (^4J 0.6 and 1.1 Hz) respectively with 15-H_3 and 10-H_β. This latter presented a vicinal coupling of 3.9 Hz with 9-H which in turn was W-type-coupled to 3-H (^4J 1.2 Hz). The C-5 proton, the X part of an ABX spin system, appeared as a double doublet (^3J 8.9 and 8.3 Hz) at δ_H 5.72 (protio form, see later). The AB part is formed by the C-6 methylene protons which resonated at δ_H 2.02 and 2.28. The low-field signal exhibited, in addition to the two- and three-bond couplings (^2J 11.1 and ^3J 8.9 Hz), a long-range coupling of 0.7 Hz with 8-H_3. Finally, the spectrum showed the presence of one aromatic chelated (3′-OH) and of three aliphatic (4-, 10-, and 13-OH) hydroxy resonances. The assignment of the 10-OH proton was straightforward as it presented a vicinal and a four-bond coupling (^3J 2.6 and ^4J 0.7 Hz) with 10-H and 9-H, respectively. The assignment of 4-OH and 13-OH protons followed from n.O.e. experiments as selective irradiation of these protons produced only a moderate saturation transfer (< 20%) to the remaining OH resonances. Specifically, irradiation of the 4-OH proton enhanced 1-H (1%), 5-H (1.5%), and 8-H_3 (0.5%), and irradiation of the 13-OH proton led to enhancement of 3-H (3%), 6-H_β (1%), 9-H (1.5%), and 12-H_β (2.5%).

The assignment of the 22 ^{13}C resonances followed from the multiplicities observed in the fully ^1H-coupled ^{13}C n.m.r. spectrum, selective low-power ^{13}C {^1H} decouplings, and chemical-shift criteria. In particular, the values of the one-bond (C,H) coupling constants exhibited by C-5 and C-6 (^1J 159 and 139 Hz, respectively), and of the three-bond (C,H) constant between C-1′ and 5-H (^3J 3 Hz) are in agreement with the presence of the cyclobutane ring C and, respectively, with the fact that a 3-chloro-4-*O*-methylorsellinate moiety is attached at C-5.

The ^1H n.m.r. spectrum of melledonal C (**11**) in [$^2H_6$]acetone recorded under conditions where the hydroxy groups are partially deuteriated displayed two resonances for both 4-OH and 10-OH signals, each resonance exhibiting the same pattern, *i.e.* 4-OH singlet, and 10-OH doublet of doublets, whereas one of the two components of 10-H showed a simplified pattern because the vicinal coupling to the 10-hydroxy proton cannot be observed for deuteriated species. Moreover, it was found that the relative ratio of the components for these protons varies with OH : OD ratios, thus permitting us to identify the direction of the isotope shift as, when OH > OD, the protio component is more intense (see Figure 2).

Recent ^1H n.m.r. studies of sugar derivatives[15–17] have demonstrated that the presence of intramolecular hydrogen bonding between partially deuteriated hydroxy groups under slow-exchange conditions is manifested by isotopically shifted hydroxy resonances (SIMPLE ^1H n.m.r.), in which the hydroxy group acting as donor exhibits a negative (to low frequency) isotope effect, and the hydroxy group acting as a hydrogen-bond acceptor exhibits a positive (to high frequency) isotope effect.

In our case the magnitude and the direction of the isotope effects for 4-OH, 10-OH, and 10-H signals were -71×10^{-4}, $+24 \times 10^{-4}$, and -81×10^{-4} p.p.m., respectively. This is consistent with 4-OH being the donor and 10-OH the acceptor as depicted in Figure 3, whereas the appearance of separate signals for 10-H results from negative isotope effect which arise when OD replaces 10-OH.

The presence of the aforementioned hydrogen bonding, as evidenced by Dreiding model inspection, indicates that rings B and C assume a conformation similar to that exhibited in the

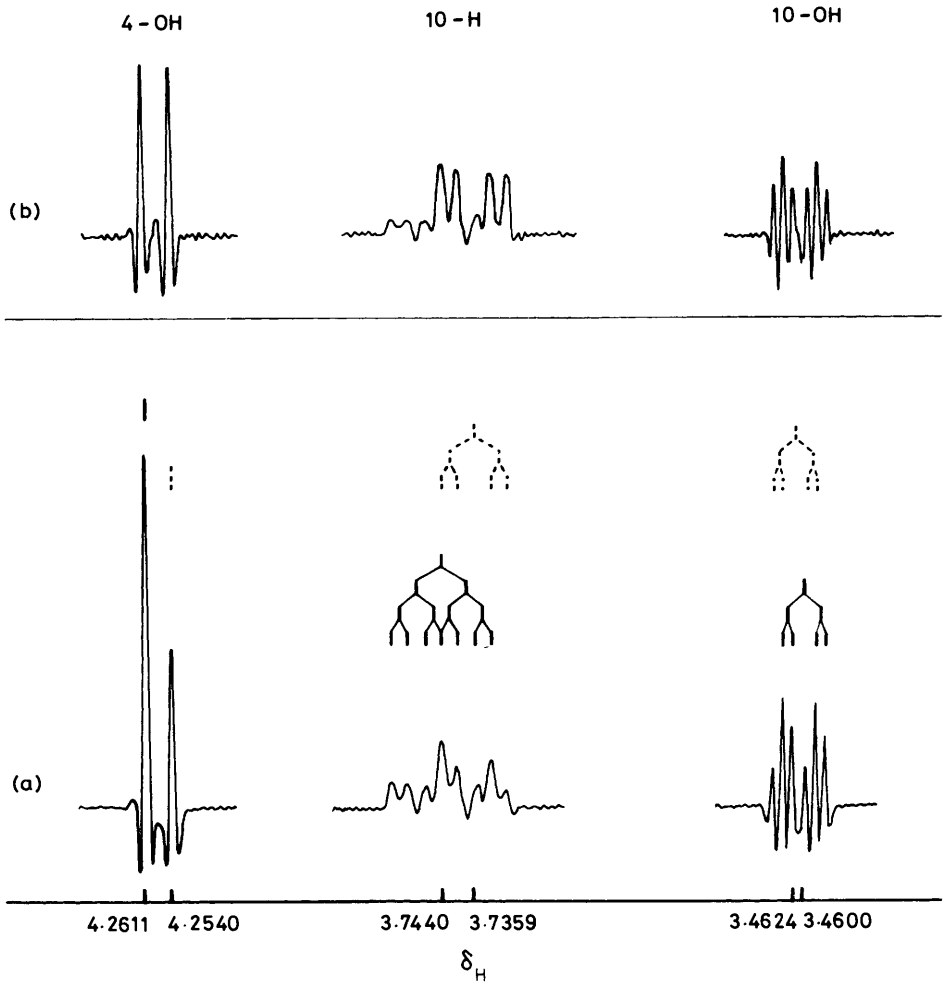


Figure 2. ^{1}H N.m.r. resonances of 4- and 10-OH and 10-H protons in [$^2H_6$]acetone at different OH:OD ratios; (a) *ca.* 3:1; (b) *ca.* 1:1. Dashed lines indicate isotope-shifted resonance signals

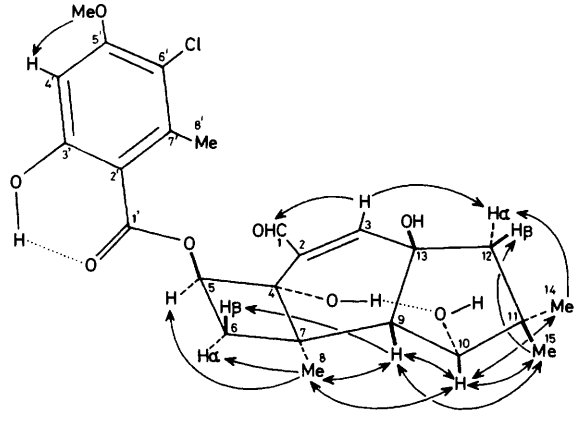


Figure 3. The n.O.e. connectivity pattern observed for melledonal C (**11**)

solid state, while the following n.O.e. experiments establish the preferred conformation of ring A. Irradiation of 9-H in [$^2H_6$]acetone + D_2O resulted in enhancement of 6-H_β (3%), 10-H (8%), 8-H_3 (1%), and 15-H_3 (3%), and irradiation of 3-H enhanced 1-H (18%) and 12-H_α (2.5%), but no n.O.e. was observed for 14-H_3. These results, as corroborated by the W-type long-range couplings between 10-H_β,12-H_β, and 12-H_α,15-H_3, indicate that ring A assumes an envelope conformation similar to that observed in the crystal in which 9-H and 15-H_3 are *cis* pseudoaxially disposed and 14-H_3 is pseudoequatorially orientated. The n.O.e. connectivity pattern for melledonal C is shown in Figure 3.

Structure Determination of Melledonals A (**9**) *and B* (**10**).—A comparison of the ^{1}H and ^{13}C n.m.r. data of melledonals A (**9**), B (**10**), and C (**11**) in [$^2H_6$]acetone (see Tables 6 and 7) indicated that these three compounds contain the same protoilludene moiety and differ from each other only in the substitution pattern of the aromatic ring D. Specifically, the difference in the ^{1}H and ^{13}C chemical shifts for analogous resonances is within 0.07 and 0.7 p.p.m., respectively, and the difference in (H–H) coupling constants is within 0.2 Hz.

Melledonal B (**10**), $C_{23}H_{27}ClO_8$, differs in molecular weight by 14 mass units from melledonal C (**11**), this fact suggesting that the ring D of the metabolite contains an 5′-hydroxy function in place of the 5′-OMe group. Methylation of melledonal B with CH_2N_2 afforded a compound which was found to be identical with melledonal C, thus identifying the structure of melledonal B as (**10**).

Melledonal A (**9**), $C_{23}H_{28}O_8$, differs by 49 mass units from melledonal C (**11**). This finding, in conjunction with the

aforementioned n.m.r. evidence, suggests the 6′-chlorine atom and the 9′-H_3 group of melledonal C are replaced by hydrogen atoms. This supposition was confirmed by the ^1H n.m.r. spectrum of melledonal A in $CDCl_3$ which exhibited for ring D an AB spin system (δ_H 6.24 and 6.14: ^3J 2.6 Hz) attributable to two *meta*-coupled protons, besides two hydroxy groups, one of which was chelated (3′-OH), and one aromatic methyl group. Moreover irradiation of the methyl group in an n.O.e. experiment in [$^2H_6$]acetone + D_2O enhanced only one of the two aromatic protons. These results and the similarity of the chemical shift of the ring D protons with those reported for the corresponding hydrogen atoms of melleolide (**1**)[5] ($\Delta\delta < 0.03$ p.p.m.) enables us to propose structure (**9**) for melledonal A.

This compound should thus possess the same structure as melledonal whose relative configuration was determined by n.O.e. experiments carried out on the aromatic diacetate (**12**).[8] The n.O.e. connectivity pattern evidenced, among others, the presence of large n.O.e.s between 3-H,12-H_α, (10%) and 3-H,14-H_3 (12.1%), and of a sizeable n.O.e. between 10-H,12-H_β (3.7%), whereas no n.O.e.s were observed between 9-H,8-H_3 and 9-H,15-H_3.

However, in our hands, both melledonal A (**9**) and its aromatic diacetate (**12**), obtained by treating (**9**) with pyridine–Ac_2O, gave n.O.e. enhancements in [$^2H_6$]acetone + D_2O and in $CDCl_3$, respectively, different from those reported,[8] but analogous to those exhibited by melledonal C and hence entirely consistent with the proposed envelope conformation for ring A. Specifically, n.O.e. experiments carried out on compound (**12**) (see Figure 4) showed the presence of spatial interactions between 8-H_3 and 9-H (4%), 9-H and 15-H_3 (11%), and 3-H and 12-H_α (5%).

No enhancements were observed between 3-H and 14-H_3,

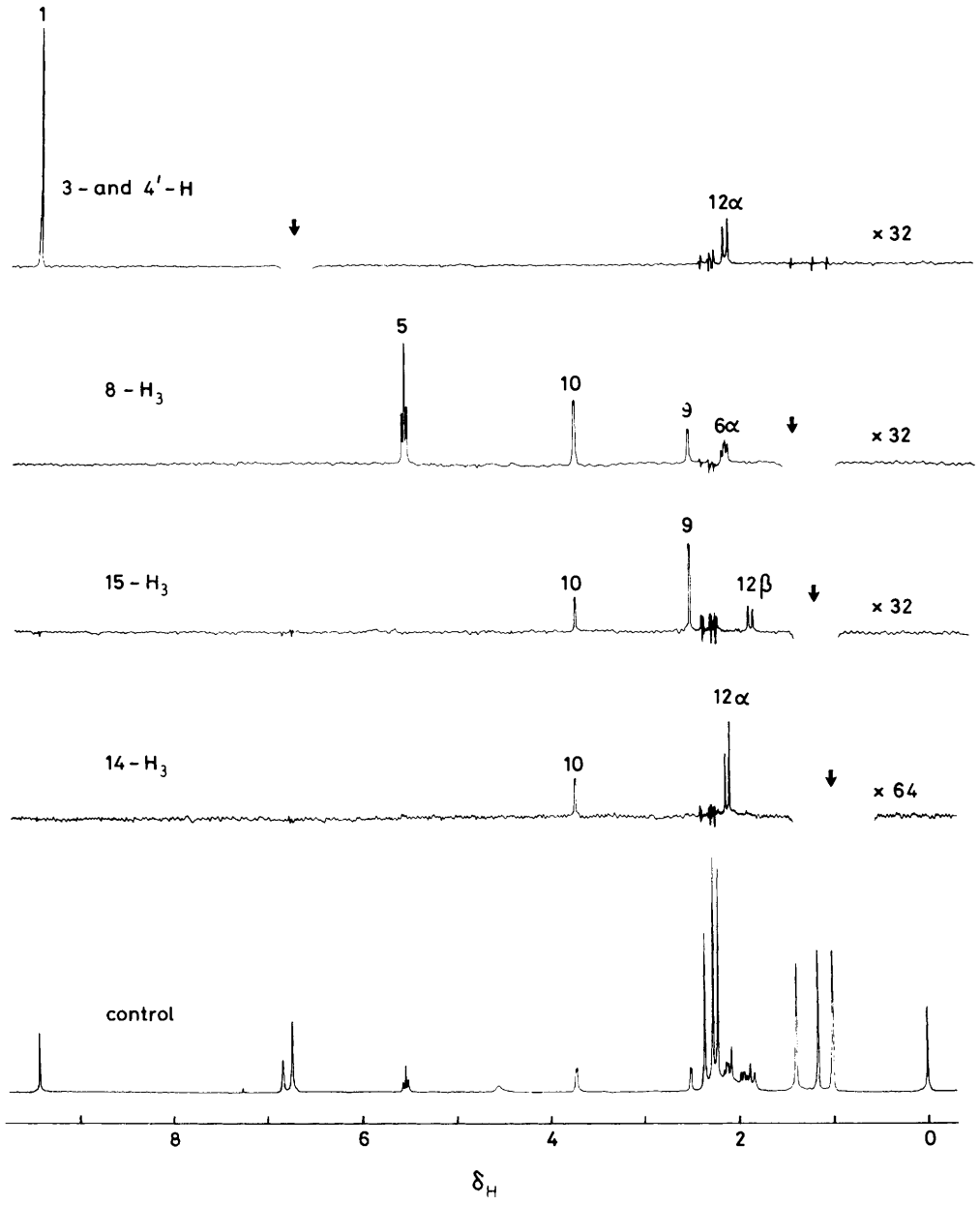


Figure 4. Control spectrum and n.O.e. difference spectra for some irradiations of the diacetate of melledonal A (**12**) in $CDCl_3$. Irradiated protons are indicated at the left and marked by an arrow, the control spectrum is broadened by 1.0 Hz

and 10-H and 12-H_β, this being in agreement with a distance of 5.0 and 4.0 Å, respectively, between these protons determined by *X*-ray analysis in the closely related melledonal C (**11**).

This different behaviour cannot be fully rationalized as the solvent used for the n.O.e. experiments was not reported,[8] but the possibility cannot be excluded that the cyclopentane ring A may adopt a different conformation in certain conditions.

Finally the application of the SIMPLE ^{1}H n.m.r. method in [$^2H_6$]acetone to melledonal A (**9**) and its diacetate (**12**) resulted in isotopic effects for 4-OH, 10-OH, and 10-H similar to those exhibited by melledonal C (**11**), *viz.* -67×10^{-4}, $+24 \times 10^{-4}$, -80×10^{-4} and -67×10^{-4}, $+22 \times 10^{-4}$, -70×10^{-4} p.p.m., respectively. This evidence reveals the presence of the intramolecular hydrogen bonding between the proton of 4-OH and the oxygen atom of 10-OH in these compounds too.

Determination of the Absolute Configuration at C(**13**) *of Melleolides C* (**6**) *and D* (**7**).—In a previous work,[2] we reported on the isolation and structure elucidation of two $\Delta^{2,3}$ protoilludene metabolites, melleolides C (**6**) and D (**7**), based mainly on ^{1}H and ^{13}C n.m.r. studies. The relative configuration, except for that at C(13), was also determined. To clarify this issue, we treated melledonal C (**11**) with $NaBH_4$, obtaining a compound identical with melleolide D (**7**). This indicates that melleolide D has the same absolute configuration as melledonal C and, consequently, it is reasonable to conclude that melleolide C, which was shown to differ from melleolide D only in the absence of the 6′-chlorine atom, possesses the same absolute configuration.

Experimental

M.p.s are uncorrected. U.v. spectra were measured for solutions in 95% EtOH on a JASCO Uvidec-510 spectrophotometer. I.r. spectra were recorded with a Perkin-Elmer 177 instrument. Flash chromatography was performed with Merck silica gel (0.040—0.063 mm), and t.l.c. with Merck HF_{254} silica gel. Mass spectra were taken on a VG-ZAB2 instrument at 70 eV. ^{1}H (300.13 MHz) and ^{13}C (75.47 MHz) N.m.r. spectra were recorded on a Bruker CXP-300 spectrometer. Chemical shifts are in p.p.m. (δ) from $SiMe_4$ as internal standard. N.O.e. difference spectra were obtained by substracting alternatively right-off resonance-free induction decays (FIDS) from right-on resonance-induced FIDS. N.O.e. values reported in the text has only qualitative significance.

Isolation and Purification of Metabolites (**9**)—(**11**).—Cultures of *Armillaria mellea*[2] were grown in daylight at 24 °C for 4 weeks in 50 Roux flasks containing malt–peptone–glucose–glycerine–agar medium (20:4:30:10:15 g l^{-1}), at pH 7. The preinoculum was grown in shaken Erlenmeyer flasks (300 ml) containing a liquid medium (malt–glucose–yeast; 10:30:10 g l^{-1}) (50 ml) for 5 days. The mycelium was extracted twice with EtOAc and the collected extracts were dried (Na_2SO_4) and evaporated to give a mixture of crude metabolites. The mixture was chromatographed on a column of flash silica gel with hexane–EtOAc (2:1) as eluant, and purified further by preparative t.l.c. (p.l.c.) with the same solvent or CH_2Cl_2–MeOH (15:1), to yield the three melledonals in the following order of descending R_F values: melledonal C (120 mg), B (50 mg), and A (70 mg).

Melledonal A (**9**), crystallized from EtOAc–hexane as white crystals, m.p. 212—214 °C (lit.,[8] 136—137 °C) (Found: C, 63.7; H, 6.5. $C_{23}H_{28}O_8$ requires C, 63.88; H, 6.53%); $[\alpha]_D$ +102° (*c* 0.2 in MeOH) (lit.,[8] +195° in MeOH); $\lambda_{max.}$ 214, 260, and 268 nm (ε 27 500, 13 800, and 5 700); $\nu_{max.}$(KBr) 3 400 (OH), 1 685 (unsatd. aldehyde), and 1 640 cm^{-1} (aryl ester); *m/z* 414 ($M^+ - 18$), 238, 220, and 151 [Ar(OH)$_2$MeCO]$^+$ (base peak); ^{1}H and ^{13}C n.m.r. data are reported in Tables 6 and 7, respectively.

Acetylation of compound (**9**). Melledonal A (**9**) (30 mg), Ac_2O (1.5 ml), and pyridine (0.5 ml) were left for 3 h at 0 °C. The solution was poured onto ice, and the resulting precipitate was purified by p.l.c. with CH_2Cl_2–MeOH (30:1) as developer to give the diacetate (**12**) as a glassy solid (from acetone–hexane), m.p. 92—95 °C; $[\alpha]_D$ +79° (*c* 0.8 in $CHCl_3$). For this compound the following data are reported:[8] oil; $[\alpha]_D$ +20.9° (*c* 0.2 in $CHCl_3$). ^{1}H N.m.r. data are reported in Table 6.

Melledonal B (**10**) crystallized from EtOAc–hexane as white crystals, m.p. 228—232 °C (Found: C, 59.0; H, 5.8; Cl, 7.7. $C_{23}H_{27}ClO_8$ requires C, 59.16; H, 5.83; Cl, 7.59%); $[\alpha]_D$ +101° (*c* 0.1 in MeOH); $\lambda_{max.}$ 213, 257, and 306 nm (32 600, 11 300, and 6 000); $\nu_{max.}$(KBr) 3 400 (OH), 1 705 (unsatd. aldehyde), and 1 640 (ester) cm^{-1}; *m/z* 466/468 (M^+), 448/450 ($M^+ - 18$), and 185/187 [Ar(OH)$_2$ClMeCO]$^+$. ^{1}H and ^{13}C N.m.r. data are reported in Tables 6 and 7, respectively.

Melledonal C (**11**), white crystals from EtOAc–hexane, had m.p. 200—205 °C (Found: C, 60.1; H, 6.1; Cl, 7.3. $C_{24}H_{29}ClO_8$ requires C, 60.06; H, 6.09; Cl, 7.38%); *m/z* 480/482 (M^+), 462/464 ($M^+ - 18$), 238, 220, and 199/201 [Ar(OH)(OMe)-ClMeCO]$^+$. ^{1}H and ^{13}C N.m.r. data are reported in Tables 6 and 7, respectively.

Biological Tests.—Antibacterial activity was tested with paper disks (6 mm diam.), soaked with compounds (**9**), (**10**), or (**11**) (100 μg), which were inoculated in suitable sterilized agar, cooled at 45 °C, and poured into Petri dishes with *Bacillus cereus* (ATCC 10702), *B. subtilis* (ATCC 6633), and *Escherichia coli* (ATCC 10536) as test micro-organisms. The compounds exhibited activity against *B. cereus* and *B. subtilis* and were inactive against *E. coli*.

Crystal Structure Determination of Melledonal C (**11**).—Transparent crystals, prismatic in habit, and stable to air.

Crystal data: $C_{24}H_{29}ClO_8$; M = 480.941. Orthorhombic, a = 21.273(6), b = 15.440(5), c = 7.151(2) Å (by least-squares refinement of 2θ values of 30 reflexions with $2\theta \geqslant 40°$), V = 2 348(1) Å^3, space group $P2_12_12_1$, Z = 4, $F(000)$ = 1 016, λ(Cu-K_α) = 1 5419 Å, μ(Cu-K_α) = 18.413 cm^{-1}.

Data collection and processing. A crystal of approximate dimensions 0.35 × 0.25 × 0.20 mm was chosen for *X*-ray analysis. A PW 1100 diffractometer, ω/2θ mode, with scan width of 1.00° and scan speed of 2.1° min^{-1} was used; two background counts were measured at each side of the peak for half the peak-measuring time and the values were averaged. 2 Standard reflexions ($\bar{3}$,4,1 and 3,$\bar{4}$,1) were measured every 2 h to check crystal centring and decay. Graphite-monochromated Cu-K_α radiation was used. 4 023 Reflexions ($2° \leqslant \theta \leqslant 60°$: 2 016 $+h$, $+k$, $+l$; 2 007 $-h$, $-k$, $-l$, 1 450 of which were non-Friedel-reflexions), resulting in 3 103 with $I \geqslant 2.5\sigma(I)$. There was no significant decay, and no absorption or extinction correction was applied.

Structure analysis and refinement. The structure was solved by direct methods (MULTAN),[18] locating 26 out of 33 non-hydrogen atoms. The remaining atoms were found by successive Fourier difference maps. Atomic positions were refined by block-diagonal least-squares (Cruickshank[19] weighting scheme) with anisotropic temperature factors; initially only the real component of the atomic scattering factors was used.[20]

The hydrogen-atom contributions were taken into account but not refined, idealized positional parameters (C–H 1.08 Å) being used throughout, with the exception of the hydroxy-group hydrogens which were located by Fourier difference maps.

At the end of the refinement the imaginary contributions to the anomalous dispersion effect for Cl, O, and C atoms were

taken into account. At convergence, with positive $i\Delta f_j''$s[20] the values of R and R_w were respectively 0.056 and 0.063, while with negative $i\Delta f_j''$s $R = 0.062$ and $R_w = 0.070$. The value of 1.093 found for the R_{2w} ratio indicates, at a high level of significance ($\alpha < 0.995$), that the correct configuration is the one refined (positive $i\Delta f_j''$s).*

Acknowledgements

We thank Professor A. Albinati for helpful discussions and Ms E. Di Modugno for skilful technical assistance.

---

* *Supplementary data* (see section 5.6.3 of Instructions for Authors, in the January issue). Anisotropic temperature factors, H-atom co-ordinates, and least-squares planes have been deposited at the Cambridge Crystallographic Data Centre.

References

1 Part 18, A. Arnone, G. Assante, G. Nasini, H. J. Roeijmans, and G. W. Van Eijk, *Phytochemistry*, 1987, **25**, 1739.
2 A. Arnone, R. Cardillo, and G. Nasini, *Phytochemistry*, 1986, **25**, 471.
3 A. Arnone, R. Cardillo, and G. Nasini, 1st International Symposium on Organic Chemistry of Medicinal Natural Products, Shanghai, China, 1985, B-033.
4 A. Arnone, R. Cardillo, G. Nasini, and G. Assante, 15th Convegno Nazionale della Divisione di Chimica Organica, Sirmione, Italy, 1985, p. 256.
5 S. L. Midland, R. R. Izac, R. M. Wing, A. I. Zaki, D. E. Munnecke, and J. J. Sims, *Tetrahedron Lett.*, 1982, **23**, 2515.
6 D. M. X. Donnelly, F. Abe, D. J. Coveney, N. Fukuda, J. O'Reilly, J. Polonsky, and T. Prangé, *J. Nat. Prod.*, 1985, **48**, 10; D. M. X. Donnelly, D. J. Coveney, N. Fukuda, and J. Polonsky, *ibid.*, 1986, **49**, 111.
7 Y. Junshan, C. Yuwu, F. Xiaozhang, Y. Dequan, and L. Xiaotian, *Planta Med.*, 1984, **50**, 288.
8 D. M. X. Donnelly, D. J. Coveney, and J. Polonsky, *Tetrahedron Lett.*, 1985, **26**, 5343.
9 D. M. X. Donnelly, S. Sanada, J. O'Reilly, J. Polonsky, T. Prangé, and C. Pascard, *J. Chem. Soc., Chem. Commun.*, 1982, 135.
10 D. M. X. Donnelly, J. Polonsky, T. Prangé, G. Snatzke, and U. Wagner, *J. Chem. Soc., Chem. Commun.*, 1984, 222.
11 F. H. Allen, *Acta Crystallogr., Sect. B*, 1984, **40**, 64.
12 J. F. Chiang and S. H. Bauer, *J. Am. Chem. Soc.*, 1969, **91**, 1898.
13 C. Altona, H. J. Geise, and C. Romers, *Tetrahedron*, 1968, **24**, 13.
14 G. A. Jeffrey and J. Mitra, *Acta Crystallogr., Sect. B*, 1983, **39**, 469.
15 J. C. Christofides and D. B. Davies, *J. Chem. Soc., Chem. Commun.*, 1982, 560.
16 J. C. Christofides and D. B. Davies, *Magn. Reson. Chem.*, 1985, **23**, 582.
17 J. C. Christofides and D. B. Davies, *J. Chem. Soc., Chem. Commun.*, 1985, 1533.
18 P. Main, S. L. Fiske, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, MULTAN, a System of Computer Programs for the Automatic Solution of Crystal Structures for X-ray Diffraction Data, Universities of York, England, and Louvain, Belgium, 1980.
19 D. W. J. Cruickshank, 'Crystallographic Computing,' ed. F. R. Ahmed, Copenhagen, Munksgaard, 1970, p. 187.
20 D. Rogers, *Acta Crystallogr., Sect. A*, 1981, **37**, 734.

Received 2nd March 1987; *Paper* 7/377