Secondary Mould Metabolites. Part 34.[1] Isolation and Structure Elucidation of Illudosin, a Novel Sesquiterpene from *Clitocybe illudens* using One and Two Dimensional NMR Techniques

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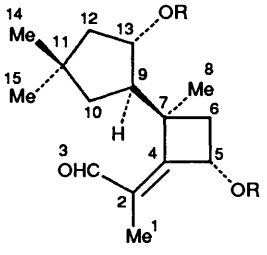
The structure of illudosin **1**, a novel sesquiterpene isolated from the still liquid cultures of *Clitocybe illudens* (= *Omphalotus olearius*), has been asssigned on the basis of ^1H and ^{13}C NMR spectroscopic studies, including ^1H–^{13}C heteronuclear correlation spectra (HETCOR and COLOC). The preferred conformation and the absolute configuration of the metabolite were deduced from NOE experiments and the application of the exciton chirality method on the dibenzoate **2**. Illudosin may be regarded as an intermediate in the biosynthesis of the fomannosin **3**.

During our continuing searches for biologically active metabolites produced by Basidiomycetes fungi,[1–3] we reported the isolation of the sesquiterpenes illudins A–E[1,3] and illudalenol[3] from cultures of *Clitocybe illudens* (= *Omphalotus olearius*). From this fungus we have now isolated a new sesquiterpene, illudosin **1**, which could be a possible biosynthetic precursor of fomannosin **3**, a metabolite implicated in the phytopathogenic activity of the wood rot fungus *Fomes annosus*.[4,5] We present here the structural elucidation of illudosin **1**.

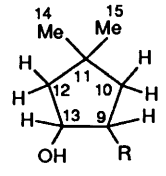


1; R = H
2; R = COPh

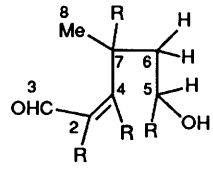
The molecular formula of illudosin **1**, $[\alpha]_D$ +52.4 10^{-1} deg cm^2 g^{-1} (*c* 0.2, $CHCl_3$), was established to be $C_{15}H_{24}O_3$ by elemental analysis and by chemical ionisation mass spectrometry (MH^+, 253), the strong peaks observed at *m/z* 235 and 217 being due to the ready loss of two molecules of water from the molecular ion. The metabolite showed IR absorptions at ν/cm^{-1} 3380 (OH) and 1680 (α,β-unsaturated CO) and UV absorptions at λ/nm 245 (ε 16 400).

The ^1H NMR spectrum (see Table 1) contained signals attributable to one aldehydic proton (3-H) and four tertiary methyl groups (1-, 8-, 14- and 15-H_3). In addition, it revealed the presence of two sequences such as –C(5)HOH–C(6)H_2– and –C(10)H_2–C(9)H–C(13)HOH–C(12)H_2–, the structures of which were readily determined from the observed vicinal coupling constants (^3J/Hz 4.7–11.8) and confirmed by selective homodecoupling experiments. The broad-band ^1H decoupled ^{13}C NMR spectrum of illudosin **1** showed the presence of 15 carbon resonances, the multiplicities of which were obtained by DEPT (distortionless enhancement by polarization transfer) experiments and analysis of the ^1H-coupled ^{13}C NMR spectrum. Three of them (C-2, C-3 and C-4) were assigned to the carbons of a fully substituted α,β-unsaturated aldehydic moiety while the remaining resonances were assigned to four methyl, three methylene, three methine, two of which are oxygen-bearing, and two quaternary sp^3-hybridized carbon atoms.

The ^1H–^{13}C heteronuclear correlation (HETCOR) spectrum of illudosin **1** shown in Fig. 1 enabled us to correlate the signals of all the proton-bearing carbon atoms with specific proton resonances[6] while the long-range ^1H–^{13}C heteronuclear correlation (COLOC) spectrum shown in Fig. 2 was used to observe ^1H–^{13}C couplings *via* two and three bonds.[7] Thus, the cross-peaks observed in Fig. 2 for both the 14- and 15-methyl protons with C-11, together with the cross-peaks observed for each methyl carbon with the other methyl protons, require the presence of a *gem*-dimethyl group located at C-11. Moreover, the fact that the *gem*-dimethyl protons presented additional cross-peaks with C-10 and C-12 is consistent only with the presence in illudosin **1** of a cyclopentane ring incorporating C-11 as shown in the part structure **A**. Similar correlations observed between the 8-methyl protons and C-4, C-6, C-7 and C-9 not only indicate the part structure **B** but also that C-7 must be linked to the C-9 carbon of the cyclopentane ring.



A



B

The magnitude of the one-bond ^1H–^{13}C coupling constants exhibited by C-5 and C-6 is very similar to that observed for the corresponding carbons of the model cyclobutanol[2] (^1J/Hz 148 and 135) and greater than that exhibited by the methylene carbons of the cyclopentane ring. It follows that C-4, C-5, C-6 and C-7 are present in illudosin **1** as a cyclobutane moiety and that the remaining C(1)H_3 group, whose protons showed cross-peaks with C-2, C-3 and C-4 (see Fig. 2) and a homoallylic coupling of 1.3 Hz with H-5, is located at C-2.

The NOE enhancements shown in Fig. 3 and Table 2, permitted us to establish the configuration of the C(2)=C(4) double bond and to assign as 5*S*, 7*R*, 9*R*, 13*S* the relative configuration at the chiral carbons, having assumed that C-7 has the *R*-configuration. Specifically, the NOEs observed between 8-H_3, assumed as being α with respect to the cyclobutane ring, and 3-H (9%), but not with 1-H_3, and between 5-H and 1-H_3 (0.5%), but not with 3-H, indicated the *E*-configuration of the double bond while the NOEs observed

Table 1 ^1H and ^{13}C NMR data for compounds **1** and **2** in [$^2H_6$]-acetone

	δ_H			J/Hz		**1**		
Proton	**1**	**2**	J(H,H)	**1**	**2**	Carbon	δ_C	^1J(C,H)/Hz[a]
1	1.73	1.70	1,5	1.3	1.4	1	9.97	128
3	9.92	10.06	5,6α	6.0	5.6	2	133.51	
5	4.72	5.93	5,6β	8.8	8.7	3	191.37	172
6α	1.77	2.10	5,5-OH	7.8		4	174.52	
6β	2.53	2.85	6α,6β	12.0	13.0	5	67.17	148
8	1.60	1.67	9,10α	7.5	7.5	6	40.02	136
9	2.34	3.08	9,10β	11.8	11.8	7	45.99	
10α	1.53	1.78	9,13	6.5	6.2	8	29.82	126
10β	1.16	1.49	10α,10β	12.8	12.6	9	56.70	126
12α	1.50	1.65	10α,12α	1.7	2.1	10	44.10	126
12β	1.76	2.11	10β,15	0.7	0.7	11	36.64	
13	3.94	5.21	12α,12β	13.0	14.2	12	51.99	127
14	1.00	1.15	12α,13	4.7	3.0	13	75.43	142
15	1.09	1.20	12β,13	7.8	8.2	14	30.87	124
5-OH	4.60	—[b]	12β,15	0.7	0.7	15	29.94	124
13-OH	3.67	—[b]	13,13-OH	6.5				

[a] Long-range (C,H) couplings: ^3J(C-1, 3-H) 3.5, ^2J(C-2, 3-H) 26 and ^3J(C-3, 1-H_3) 4 Hz. [b] The aromatic protons resonate between 8.2 and 7.4 ppm.

Table 2 Selected connectivities for compounds **1** and **2** established by NOE difference experiments in [$^2H_6$]-acetone

Proton irradiated	Protons affected in compound (%)	
	1[a]	**2**[b]
3	1(0.5), 8(1.5), 9(5.5)	1(0.5), 8(1.5), 9(6.5)
5	1(0.5), 6β(3.5), 10β(3)	1(1), 6β(4), 10β(4.5)
6β	5(5), 6α(29), 10β(2.5), 13(3.5)	5(7), 6α(32), 10β(4), 13(5)
8	3(9), 6α(5), 9(5), 13(3.5)	3(12), 6α(6), 9(6), 13(6)
13	6β(3), 8(1), 9(1), 10β(1), 12β(6)	6(4), 8(1), 9(1), 10(1), 12(7)
14	10α(0.5), 10β(4.5), 12α(0.5), 12β(3.5), 15(1.5)	10α(1), 10β(5.5), 12α(1), 12β(5.5)
15	9(4.5), 10α(2.5), 12α(2.5), 14(3)	9(6), 10α(4), 12α(4)

[a] Irradiation of 5- and 13-OH enhanced 1(0.5), 6α(2), 6β(0.5), 8(0.5), 9(2), 12α(2). [b] The NOE enhancements obtained by irradiation of 8-H_3 may be overestimated because of the partial irradiation of 1-H_3 and 12-Hα.

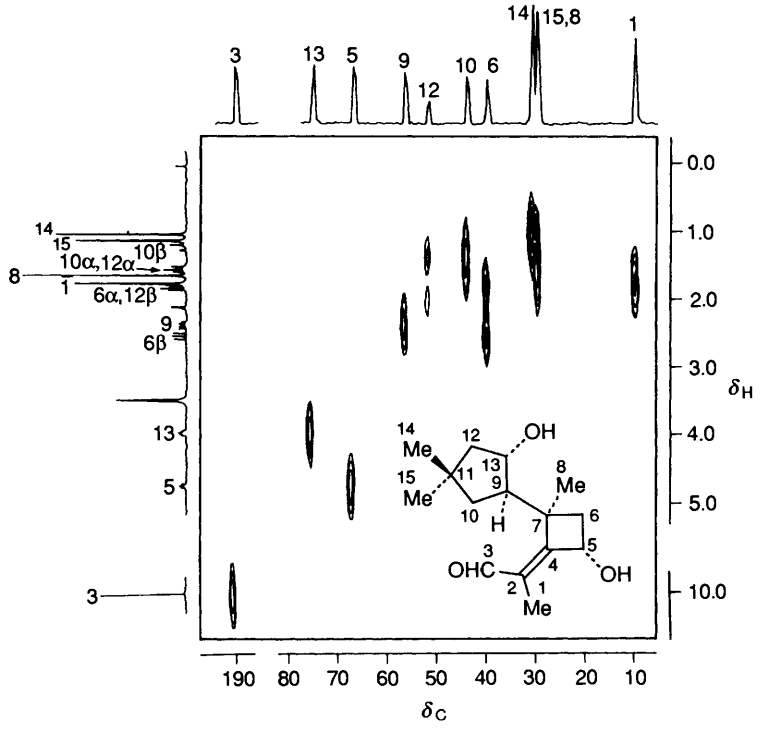


Fig. 1 HETCOR spectrum of illudosin **1** in [$^2H_6$]-acetone + D_2O, optimized for the observation of one-bond ^1H–^{13}C couplings of *ca* 148 Hz. A normal ^1H NMR spectrum is plotted along the vertical axis and the ^{13}C projection is on the horizontal axis.

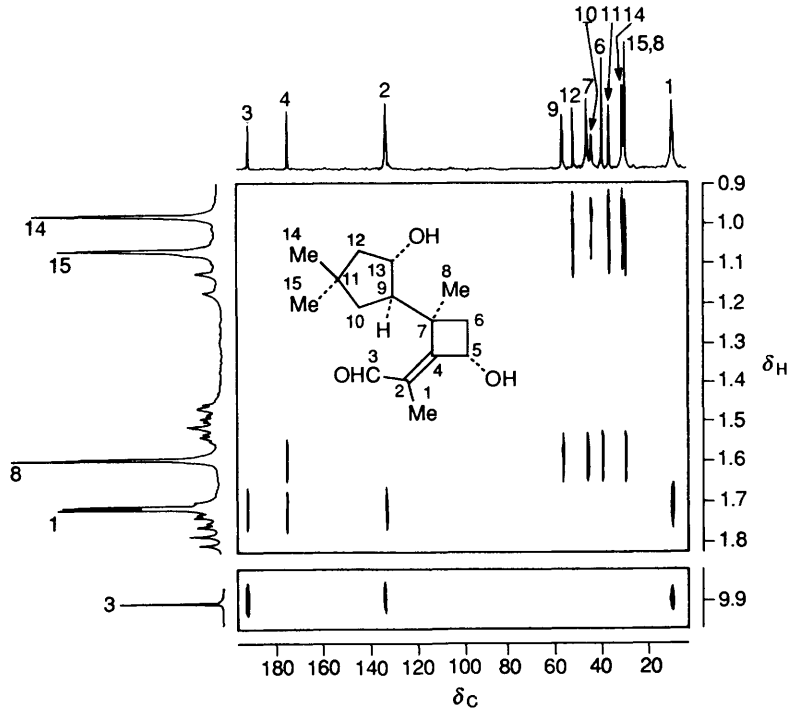


Fig. 2 Selected portion of the COLOC spectrum of illudosin **1** in [$^2H_6$]-acetone + D_2O, optimized for the observation of long-range ^1H–^{13}C couplings of *ca.* 6.3 Hz (D2 80 ms, D3 40 ms). A normal ^1H NMR spectrum is plotted along the vertical axis and the ^{13}C projection is on the horizontal axis.

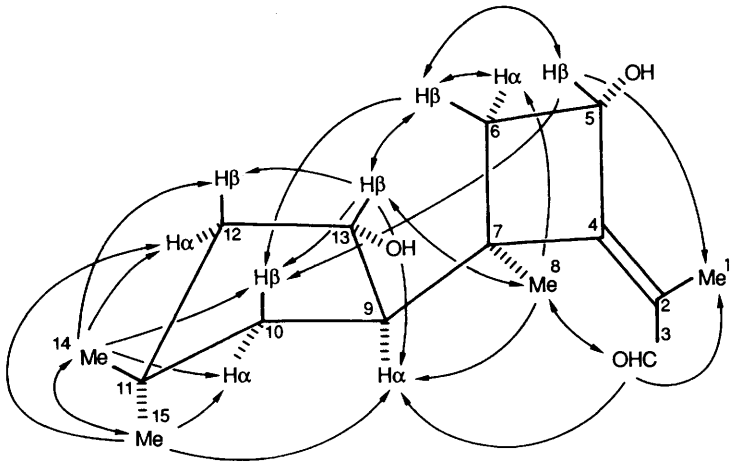


Fig. 3 Selected NOE enhancements and preferred conformation for illudosin **1**

between 8-H_3 and the 6-methylene proton at δ 1.77 (5%) (thus assigned as α) and between 5-H and 6-Hβ (3.5%), but not with 6H-α, require that 5-H is β-disposed and that C-5 has the *S*-configuration.

The NOEs observed for 9-H (4.5%) and the 10- and 12-methylene protons at δ 1.53 and 1.50 (2.5 and 2.5%) upon irradiation of 15-H_3, assumed as being α with respect to the cyclopentane ring, require that all these protons are on the same α-side of the ring while the NOE observed between 10-Hβ at δ 1.16 and 13-H (1%) implies that the latter is also β-oriented. These findings, together with the presence of W-type long-range couplings of 0.7 Hz between the 15-methyl protons and 10- and 12-Hβ, and of 1.7 Hz between 10- and 12-Hα, define the *trans*-relationship between the C-9 and C-13 substituents and indicate that the cyclopentane ring preferentially adopts the envelope-like conformation shown in Fig. 3 in which 10- and 12-Hβ are *cis*-pseudoaxially disposed. Finally, the NOEs observed between 5-Hβ and 10-Hβ (3%), between 6-Hβ and 10-Hβ (2.5%) and 13-Hβ (3%), and between 8-H_3 and 9-Hα (5%) and 13-Hβ (3.5%) suggest that the two portions of the molecule assume the preferred conformation depicted in Fig. 3, the H(9)–C(9)–C(7)–C(6) dihedral angle being *ca.* 180°. It follows that the relative configurations at C-9 and C-13 are *R* and *S* respectively, the enantiomeric ones being not compatible with the above NOE results.

The absolute configuration of illudosin **1** was determined by applying the exciton chirality method[8] to the corresponding dibenzoate **2**. In fact, this compound, which adopts a preferred conformation comparable to that of illudosin **1**, as evidenced by the ^1H–^1H couplings constants and the NOE enhancements shown in Tables 1 and 2, exhibited in the CD spectrum (Fig. 4) a positive first Cotton effect at 240 nm, indicating the right handedness of the orientation of the two benzoate groups. These data suggest the 5*S*, 13*S* configurations for compound **2** and, therefore, the 5*S*, 7*R*, 9*R*, 13*S* absolute configuration for illudosin **1**.

Biogenetic Aspects.—Studies on the biogenesis of foman-

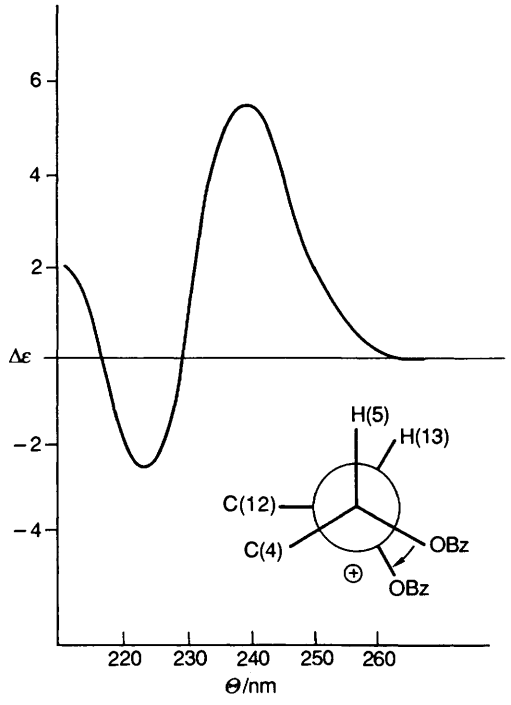
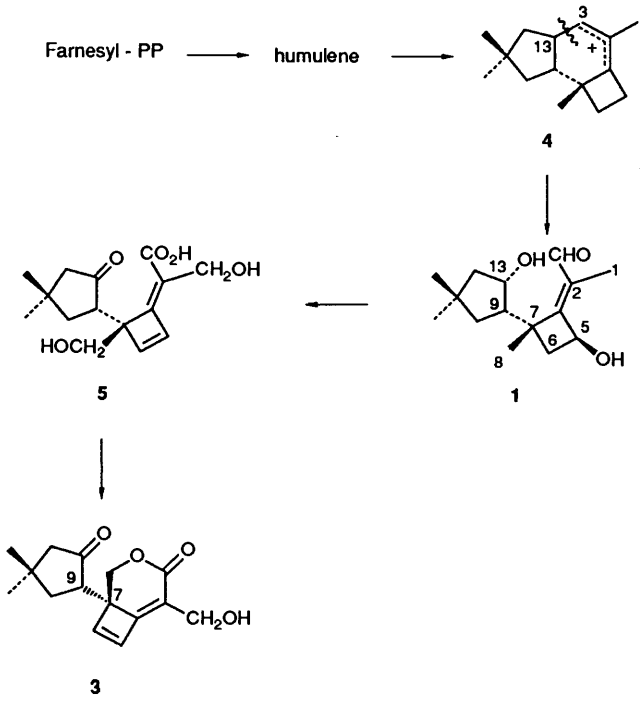


Fig. 4 The CD spectrum of compound **2**



Scheme 1

nosin **3** have shown that this metabolite arises from oxidative cleavage of the C(3)–C(13) bond of the protoilludane-type precursor **4** which, in turn, derives from farnesyl pyrophosphate *via* humulene (see Scheme 1).[9] While the cooccurrence of Δ^6-protoilludene from a fomannosin-producing strain of *Fomitopsis insularis*[10] gave further support to the proposed route, no detailed pathway has been established on the conversion of the cation **4** into fomannosin **3**.

In this context, the natural occurrence of illudosin **1**, which has the same absolute configuration[11] at C-7 and C-9 as fomannosin **3** (the change in the descriptor for the C-7 chiral centre is merely the result of the sequence rules of the Cahn–Ingold–Prelog system), is significant as it could be a key intermediate in the above-cited conversion. In fact, oxidation of the 13-OH and 2-CHO groups, hydroxylation at C-1 and C-8 and loss of water to produce the C(5)=C(6) double bond could yield the hydroxy acid **5** which would lead to fomannosin **3** by subsequent lactonization.

Illudosin **1** shows antibacterial activity against *Bacillus cereus*, *B. subtilis* and *Sarcina lutea* (50 μg/disc) but not against *Escherichia coli*; no activity was found on *Saccharomyces cerevisiae*, *Cladosporium cladosporioides* and *Chlorella vulgaris*.

Experimental

UV spectra were measured on a JASCO Uvidec-510 spectrophotometer. The IR spectrum was recorded with a Perkin-Elmer 177 instrument (liquid film). TLC and PLC were performed with Merck HF_{254} silica gel. The optical rotation was measured on a JASCO DIP-181 polarimeter and is given in 10^{-1} deg cm^2 g^{-1}. The CD spectrum was measured with a JASCO-500A dichrograph. Mass spectra were taken on a VG-ZAB2 instrument at 70 eV. NMR spectra were recorded on a Bruker AC 250L operating at 250.1 MHz for ^{1}H and 62.9 MHz for ^{13}C. Chemical shifts are in ppm (δ) from $SiMe_4$ as internal standard, J values are given in Hz. NOE difference spectra were obtained by subtracting alternatively right-off resonance-free induction decays (FIDs) from right-on resonance-induced FIDs. NOE values reported in the text have only qualitative significance. DEPT, HETCOR and COLOC spectra were performed using the DEPT, XHCORRDC and COLOC pulse sequences of the AC 250L software. Owing to the complexity of the purification procedure, we report the R_f-values in hexane–EtOAc (1:1) and CH_2Cl_2–MeOH (15:1).

Isolation and Purification of Illudosin **1**.—A strain of *Clitocybe illudens* Sacc. [= *Omphalotus olearius* Singer (CBS 164.51)] received from Centraal Bureau voor Schimmel Cultures-Baarn, was grown on a MPG (malt, peptone, glucose; 20:4:30 g dm^{-3}) liquid medium as previously described;[1,3] the EtOAc extracts (1.6 g) of the culture filtrates containing a mixture of illudins A–E[1,3] and illudalenol[3] were chromatographed on a column of flash silica gel with hexane–EtOAc (2:1) as eluent. The more polar fraction, after a further PLC purification with CH_2Cl_2–MeOH (15:1), gave illudosin **1** (10 mg).

Illudosin **1**.—This *compound* was isolated as an oil; R_f 0.2 and 0.15 (Found: C, 71.2; H, 9.4. $C_{15}H_{24}O_3$ requires C, 71.39; H, 9.59%); *m/z* (CI, isobutane) 253 (MH^+) (10), 235 [(MH^+) – 18] (100), 217 [(MH^+) – 36] (70), 189(25), 137(45), 125(64), 123(68) and 83(94). ^{13}C and ^{1}H NMR spectroscopic data are reported in Table 1.

Benzoylation of Illudosin **1**.—Illudosin **1** (15 mg) was dissolved in dry pyridine (0.3 cm^3) containing benzoyl chloride (0.03 cm^3) and the solution was kept at 0 °C for 1 h. The mixture was poured into ice–water and extracted with CH_2Cl_2. Evaporation of the solvent and PLC in hexane–EtOAc (4:1) gave the dibenzoate **2** as an oil; λ_{max} (2-methylheptane)/nm 200 and 230 (ε 30 200 and 41 400); *m/z* (CI, isobutane) 463 (MH^+); CD (*c* 2 × 10^{-1} g dm^{-3}, 2-methylheptane) 223 and 240 nm (Δε +5.5 and −2.4); ^{13}C and ^{1}H NMR spectroscopic data are reported in Table 1.

Biological Tests.—Antibacterial and antifungal activity were tested with paper disks (6 mm diam.), soaked with illudosin **1** (200, 100 and 50 μg) dissolved in EtOH which were placed in suitable culture medium, cooled at 45 °C, and poured into Petri dishes with *Bacillus cereus* (ATCC 10702), *Sarcina lutea* (DMS

348), *Escherichia coli* (IPV 287), *Bacillus subtilis* (ATCC 6633), *Saccharomyces cerevisiae* (NCYC 729), *Cladosporium cladosporioides* (IPV F167) and *Chlorella vulgaris* (Algae), as test micro-organisms. Illudosin **1** also exhibited activity on Gram (+) bacteria at concentration of 50 γ/disks.

Acknowledgements

This work was supported by Consiglio Nazionale delle Ricerche (CNR) Roma, Progetto finalizzato (Chimica Fine II).

References

1 Part 33, A. Arnone, R. Cardillo, V. Di Modugno and G. Nasini, *Gazz. Chim. Ital.*, in the press.
2 A. Arnone, R. Cardillo, V. Di Modugno and G. Nasini, *Gazz. Chim. Ital.*, 1988, **118**, 517; A. Arnone, R. Cardillo and G. Nasini, *Gazz. Chim. Ital.*, 1988, **118**, 523; A. Arnone, R. Cardillo, V. Di Modugno and G. Nasini, *J. Chem. Soc., Perkin Trans. 1*, 1989, 1995.
3 A. Arnone, R. Cardillo, G. Nasini and O. Vajna de Pava, *J. Chem. Soc., Perkin Trans. 1*, 1991, 733.
4 W. A. Ayer and L. M. Browne, *Tetrahedron*, 1981, **37**, 2199.
5 A. T. McPhail and G. A. Sim, *J. Chem. Soc. B*, 1968, 1104.
6 A. Bax, *J. Magn. Reson.*, 1983, **53**, 517; V. Rutar, *J. Magn. Reson.*, 1984, **58**, 306; J. A. Wilde and P. H. Bolton, *J. Magn. Reson.*, 1984, **59**, 343.
7 H. Kessler, C. Griesinger, J. Zarbock and H. R. Loosli, *J. Magn. Reson.*, 1984, **57**, 331; H. Kessler, C. Griesinger and J. Lantz, *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 444.
8 N. Harada and K. Nakanishi, *Circular Dichroic Spectroscopy*, O.U.P., Oxford, 1983.
9 D. E. Cane and R. B. Nachbar, *J. Am. Chem. Soc.*, 1978, **100**, 3208.
10 S. Nozoe, H. Kobayashi, S. Urano and J. Furukawa, *Tetrahedron Lett.*, 1977, 1381.
11 D. E. Cane, R. B. Nachbar, J. Clardy and J. Finer, *Tetrahedron Lett.*, 1977, 4277.

Paper 1/00996F
Received 4*th March* 1991
Accepted 4*th April* 1991