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Tropical Marine Algae. VIII†
The Structural Determination of Novel Sesquiterpenoid Metabolites from the Red Alga *Laurencia majuscula*

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Abstract

An investigation of the natural products chemistry of the red alga *Laurencia mujuscula* from the Great Barrier Reef yielded three novel metabolites, (1), (2) and (3a), possessing a new sesquiterpenoid structural type. Known metabolites (6)–(11) were also isolated and the reassignment of n.m.r. spectral values for (11) is reported.

Introduction

Although algae of the genus *Laurencia* within the family Rhodomelaceae have been extensively investigated for their natural products chemistry,[1,2] these investigations have by no means closed the book on new chemical structures from this genus. This is in part due to the variation in the nature of metabolites between species,[1] and even within the same species exposed to different environments.[2–5] It was the demonstrated richness and variety of secondary metabolites from this genus that led to the current investigation involving the re-collection and extraction of *Laurencia majuscula* (Rhodophyta, Ceramiales, Rhodomelaceae) from Geoffrey Bay, Magnetic Island. This species has been previously investigated from two locations in Great Barrier Reef water,[2] Zoe Bay, Hinchinbrook Island, and Florence Bay, Magnetic Island, and from a number of other sites within its range of distribution.[1,6]

† Part VII, *J. Nat. Prod.*, 1990, **53**, 845.

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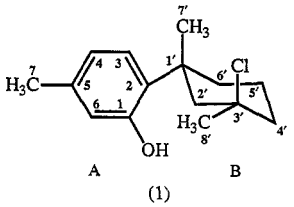
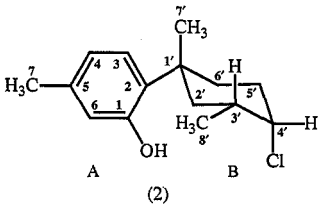
This reinvestigation resulted in the location and structural elucidation of three novel sesquiterpenoid metabolites (1)–(3) and the known sequiterpenes (6)–(11).



(1)



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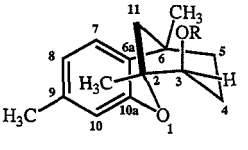

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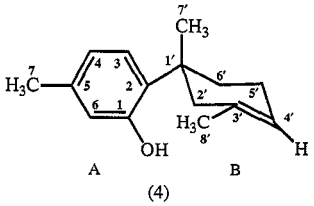
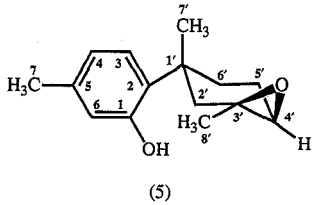
(3b) R = Ac



(4)



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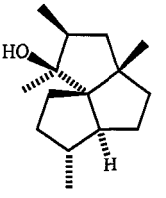
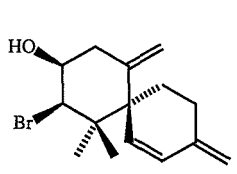


(6)



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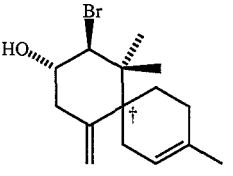

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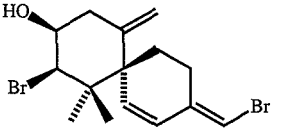

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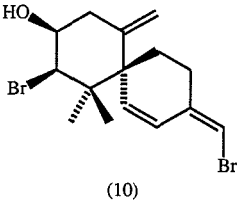

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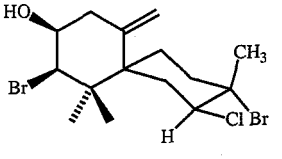

(11)

† Stereochemistry not assigned.

Results and Discussion

High-resolution mass spectrometric analysis of the least polar of the three novel compounds (1) revealed a molecular formula of $C_{15}H_{21}ClO$, this indicating five double-bond equivalents. The presence of signals in the ^{13}C n.m.r. spectrum of (1) for six sp^2 carbon atoms [115·5 (d), 120·1 (d), 124·5 (d), 126·1 (s), 136·9 (s), 155·8 ppm (s)] accounted for three degrees of unsaturation; the molecule was bicyclic. Three very deshielded proton signals in the ^1H n.m.r. spectrum of (1) in $CDCl_3$ at δ 6·68 (d, J 1·0 Hz), 6·70 (dd, J 1·0, 7·7 Hz) and 7·10 (d, J 7·7 Hz) were characteristic of a 1,2,4-trisubstituted benzene derivative, as also were absorbances in the i.r. spectrum (ν_{max} 860, 710 cm^{-1}). In conjunction with

the ^{13}C n.m.r. data, one of the substituents on the aromatic ring must be a methyl group (^{1}H δ 2·32, s), the second, a phenolic hydroxy group [^{13}C 155·8 ppm (s); ν_{max} 3622 cm^{-1}], and the last, the attachment of the second ring to the molecule.

Unambiguous assignment of proton signals to the signals for the carbon atoms to which they are attached was established by using a two-dimensional shift-correlated heteronuclear n.m.r. experiment (J 135 Hz, XHCORRD). Because of the presence of two overlapping methyl signals in the spectrum at δ 1·41 ($CDCl_3$), the sample was dissolved in C_6D_6, and the heteronuclear two-dimensional experiment was repeated. This allowed sufficient separation of the signals to unambiguously assign all proton signals to their respective carbons (Table 1).

Table 1. ^{13}C and ^{1}H n.m.r. data for (1)

Assignments are based on short-range (J 135 Hz, XHCORRD) ^{13}C–^{1}H correlations

^{13}C (ppm)	^{1}H (δ) $CDCl_3$	C_6D_6	Carbon number
19·9 (t)	1·35 (m), *eq*	1·26 (m)	5′
	1·56 (m), *ax*	1·49 (m)	
20·9 (q)	2·32 (s)	2·14 (s)	7
27·1 (q)	1·41 (s)	1·19 (s)	7′
29·2 (q)	1·41 (s)	1·24 (s)	8′
32·8 (s)			1′
38·8 (t)	1·46 (m), *ax*	1·16 (m)	4′
	1·98 (dm, J 10·9 Hz), *eq*	1·80 (dm, J 13·1 Hz)	
40·5 (t)	1·38 (m), *ax*	1·25 (m)	6′
	1·58 (d, J 12·9 Hz), *eq*	1·40 (m)	
44·1 (t)	1·58 (m), *ax*	1·15 (m)	2′
	1·79 (td, J 2·4, 13·0 Hz), *eq*	1·53 (td, J 2·3, 12·7 Hz)	
74·8 (s)			3′
115·5 (d)	6·68 (d, J 1·0 Hz)	6·79 (s)	6
120·1 (d)	6·70 (dd, J 1·0, 7·7 Hz)	6·64 (d, J 7·7 Hz)	4
124·5 (d)	7·10 (d, J 7·7 Hz)	6·98 (d, J 7·7 Hz)	2
126·1 (s)			2
136·9 (s)			5
155·8 (s)			1

A two-dimensional homonuclear correlation (COSYDQF, $CDCl_3$) experiment confirmed the presence of the 1,2,4-trisubstituted benzene ring. However, because of the overlap of a large number of signals in the ^{1}H n.m.r. spectrum, the remaining portion of (1) could not be established by only ^{1}H–^{1}H correlations. Cross peaks in the COSY spectrum of (1), however, showed a correlation between the methylene proton signal at δ 1·98 (38·8 ppm), its geminal partner at 1·46 (38·8 ppm), and the proton signals at 1·56 (19·9 ppm) and 1·79 (44·1 ppm; long range). The methylene proton signal at δ 1·79 showed cross peaks to 1·58 (44·1, 40·5 ppm) and 1·98 (38·8 ppm).

Long-range correlations from a two-dimensional heteronuclear experiment (J 10 Hz, COLOC) and selective one-dimensional heteronuclear experiments (J 7 Hz, INAPT) in $CDCl_3$ and C_6D_6 were used to confirm the assignment of the carbon skeleton. Correlations from the aromatic proton signals at δ 6·70 (120·1 ppm) and 7·10 (124·5 ppm, $CDCl_3$) and the aromatic methyl signal at 2·32 (20·9 ppm, $CDCl_3$) confirmed the proposed aromatic system, while correlations from the

aliphatic proton signals established the assignment of carbons in the saturated ring (Table 2).

Table 2. ^{13}C and ^{1}H n.m.r. correlation data for (1)

^{1}H (δ)	^{13}C–^{1}H long-range correlations
1·19[A]	32·8, 40·5, 44·1, 126·1
1·24[A]	38·8, 44·1, 74·8
1·79[B]	27·1, 29·2, 32·8, 38·8, 40·5, 74·8, 126·1
1·98[B]	19·9, 29·2, 40·5, 44·1, 74·8
2·32[C]	115·5, 120·1, 136·9
6·70[C]	20·9, 115·5, 126·1
7·10[C]	136·9, 155·8

[A] Assignments based on long-range (J 10 Hz, COLOC, C_6D_6) ^{13}C–^{1}H correlation.
[B] Assignments based on one-dimensional INAPT (J 7 Hz, $CDCl_3$) experiment.
[C] Assignments based on long-range (J 10 Hz, COLOC, $CDCl_3$) ^{13}C–^{1}H correlation.

The relative stereochemistry of the molecule was determined by n.O.e. difference spectroscopy (C_6D_6) and proton-coupling data (Table 1). Equatorial proton attributions were made for the methylene proton resonances at δ 1·79, 1·98, 1·35 and 1·58 (Table 1, $CDCl_3$). These were based on the absence of more than one large coupling ($J > 8$ Hz) in the signals, and the fact that, in general, equatorial signals are more deshielded than their axial partners. The exception to this was H 5′ (δ 1·35), which was upfield of its axial partner. The deshielded nature of the axial H 5′, and the shielded nature of the C 5′ signal (19·9 ppm), were consistent with the presence of an axial chlorine atom on C 3′. Further support for these stereochemical assignments was provided by the following n.O.e. experiments. Irradiation of the methyl signal at δ 1·19 (C_6D_6) caused an n.O.e. enhancement of the signals at 1·40 (2·5%), 1·53 (0·9%) and 6·98 (4·5%). Irradiation of the signal at δ 1·53 (C_6D_6) caused an n.O.e. enhancement of its geminal proton signal at 1·15 (6·7%) and the methyl signal 1·19 (1·8%). Irradiation of the methyl signal at δ 1·24 (C_6D_6) caused an n.O.e. enhancement of the signals at 1·53 (2·4%) and 1·80 (2·5%). Finally, irradiation of the one-proton signal at δ 1·80 (C_6D_6) caused an n.O.e. enhancement of its geminal proton partner at 1·16 (8·3%), the methyl signal at 1·24 and the methylene signal at 1·26 (5·3%). These data are consistent with the relative stereochemistry of the molecule depicted in (1), and explain the long-range $^{3}J_{H-H}$ (W-coupling) observed in the COSYDQF spectrum. Compound (1) is therefore (1′*R**,3′*R**)-2-(3′-chloro-1′,3′-dimethylcyclohexyl)-5-methylphenol.

The next more polar of the three novel compounds (2) was isomeric with (1) ($C_{15}H_{21}ClO$ by h.r.e.i.m.s.) which required five degrees of unsaturation. From ^{13}C n.m.r. spectroscopy, there were signals for six sp^2 carbons [117·8 (d), 121·0 (d), 128·8 (d), 128·8 (s), 136·8 (s), 154·6 ppm (s)], which suggested that its structure was also similar to that of (1). The presence of two overlapping carbon signals at 128·8 ppm was demonstrated by an off-resonance proton-decoupled carbon n.m.r. experiment. The presence of signals in the ^{1}H n.m.r. spectrum for three aromatic protons at δ 6·54 (s), 6·73 (d, J 7·4 Hz) and 7·12 (d, J 7·4 Hz), and absorbance in the i.r. spectrum (ν_{max} 846, 710 cm^{-1}) again indicated the presence of a 1,2,4-trisubstituted benzene ring, and led to the conclusion that the compounds differed only in the substitution pattern on the saturated ring. Substituents on

the aromatic ring were again methyl (^{1}H n.m.r. δ 2·30, s), hydroxyl (^{13}C n.m.r. 154·6 ppm, s; ν_{max} 3603 cm^{-1}) and the attachment of the second ring of the molecule (Table 3).

Table 3. ^{13}C and ^{1}H n.m.r. data for (2)

Assignments are based on short-range (J 135 Hz, XHCORRD) and long-range (J 7 Hz, INAPT; J 10 Hz, COLOC) ^{13}C–^{1}H correlations

^{13}C (ppm)	^{1}H (δ)	^{13}C–^{1}H long-range correlations[A]	Carbon number
19·9 (q)	1·01 (d, J 6·6 Hz)	33·6, 38·5, 67·2	8′
20·5 (q)	2·30 (s)	117·8, 121·0, 136·8	7
30·2 (t)	1·82 (m), *ax*		6′
	2·55 (br d), *eq*	**30·3, 32·0, 38·5, 38·9, 67·2, 128·8**	
30·3 (q)	1·32 (s)	30·2, 38·5, 128·8	7′
32·0 (t)	1·95 (m), *ax*	33·6, 38·9	5′
	2·00 (m), *eq*		
33·6 (d)	1·85 (qd, J 6·6, 13·3 Hz), *ax*		3′
38·5 (t)	1·55 (dd, J 14·1, 13·3 Hz), *ax*	**38·9, 67·2, 128·8**	2′
	2·43 (br d, J 14·1 Hz), *eq*	**19·9, 30·3, 33·6, 38·9, 67·2, 128·8**	
38·9 (s)			1′
67·2 (d)	4·22 (br s), *eq*	**19·9, 30·2, 32·0, 33·6, 38·5**	4′
117·8 (d)	6·54 (br s)	20·5, 121·0	6
121·0 (d)	6·73 (br d, J 7·4 Hz)	117·8, 128·8	4
128·8 (s)			2
128·8 (d)	7·12 (d, J 7·4 Hz)	136·8, 154·6	3
136·8 (s)			5
154·6 (s)			1

[A] Assignments from INAPT experiments are printed in **bold**; assignments from COLOC experiments are printed in light type.

Proton double-resonance experiments and a two-dimensional homonuclear correlation (COSYDQF) experiment allowed the nature of the remaining fragment to be determined. Irradiation at δ 1·55 collapsed the signal at 2·43 to a doublet and simplified the signal at 1·85. Similarly, irradiation at δ 1·85 collapsed the signals at 1·55 and 2·43 to doublets and the methyl doublet at 1·01 to a singlet. Irradiation at δ 2·43 collapsed the signal at 1·55 to a doublet, and sharpened the signal at 1·85. Irradiation at δ 1·01 collapsed the signal at 1·85. Irradiation at δ 1·82 removed the large coupling from the signal at 2·55 while irradiation at 2·55 affected the signals at 2·00, 1·95 and 1·82. These correlations were confirmed by cross peaks linking these signals in the COSYDQF experiment. Further cross peaks in this spectrum allowed the establishment of the remaining portion of the second ring.

Unambiguous assignment of protons to their attached carbons was established by a two-dimensional shift-correlated heteronuclear n.m.r. experiment (J 135 Hz, XHCORRD) (Table 3). Long-range correlations from COLOC (J 10 Hz) and **selective INAPT** experiments (J 7 Hz) confirmed the assignment of the carbon skeleton (Table 3).

The relative stereochemistry of the molecule was determined from n.O.e. experiments and proton coupling data (Table 3). The proton on the chlorine-

bearing C 4′ (δ 4·22) appeared as a broad singlet; clearly the chlorine substituent was axial. The C 3′ methine signal contained one large (J 13·3 Hz) coupling; this proton was also axial. The remaining proton assignments were based on coupling information. N.O.e. spectroscopy confirmed these assignments as follows. Irradiation of the methyl signal at δ 1·01 (19·9 ppm) enhanced signals of the protons at 1·55 (1·4%), 1·85 (2·8%), 2·43 (1·2%) and 4·22 (1·6%), while irradiation of the methyl signal at 1·32 (30·3 ppm) enhanced the signals at 1·85 (1·6%), 2·43 (1·0%) and 2·55 (0·9%). Similarly, irradiation at δ 4·22 caused enhancement of the signals at 1·01 (1·8%), 1·85 (6·8%) and 1·95 (3·7%). These data establish the relative configuration of the molecule depicted in (2). Compound (2) is therefore (1′R*,3′R*,4′S*)-2-(4′-chloro-1′,3′-dimethylcyclohexyl)-5-methylphenol.

The most polar of the three novel compounds (3a) had the molecular formula $C_{15}H_{20}O_2$ by h.r.e.i.m.s., which corresponded to six double-bond equivalents. Because initially only 14 signals were observed in the ^{13}C n.m.r. spectrum ($CDCl_3$) of (3a), the sample was dissolved in C_6D_6 and the experiment repeated. This permitted the observation of the quaternary carbon signal at 76·8 ppm. The presence of six sp^2 carbon signals in the ^{13}C n.m.r. spectrum of (3a), and signals for three aromatic protons in the ^1H n.m.r. spectrum, again suggested the presence of a 1,2,4-trisubstituted aromatic ring. Thus the molecule must be tricyclic. Substituents on the aromatic ring were a methyl group [δ 2·27 (21·0 ppm)], an oxygen function [^{13}C 154·6 ppm, (s)], and the attachment of the second ring.

A two-dimensional homonuclear n.m.r. correlation experiment (COSYDQF) confirmed the presence of a 1,2,4-trisubstituted aromatic ring, and allowed a major portion of the remaining fragment to be established. Unambiguous assignment of protons to their attached carbons was established by a two-dimensional shift-correlated heteronuclear n.m.r. experiment (J 135 Hz, XHCORRD) (Table 4).

Long-range correlations from further two-dimensional heteronuclear n.m.r. experiments (J 10 Hz, COLOC), and one-dimensional selective INAPT experiments (J 7 Hz), were used to confirm the assignment of the carbon skeleton. The connectivities established from the aromatic protons and the aromatic methyl group further substantiate the presence of the substituted benzene ring, while correlations from the aliphatic proton signals confirmed the nature of the saturated ring (Table 4). Connectivity could not be observed between the quaternary oxygenated carbons (76·8 ppm in the cyclohexane ring and 154·6 ppm in the aromatic ring). The only possible connection between these two carbons, however, is through an ether linkage creating the third ring. This was substantiated by the observation of a C–O–C absorption band (1056 cm^{-1}) in the i.r. spectrum of (3a).

The relative stereochemistry of the molecule was determined by n.O.e. experiments and proton coupling data (Table 4). The ether formation between the benzene and cyclohexane rings results in a conformational change in the cyclohexane ring. The ring adopts the alternative chair conformation, in which the hydroxy group is axial as revealed by the small couplings in the hydroxymethine proton (H 3) signal. The details of this new conformation, which is consistent with the coupling constant data, are depicted in (3a). Irradiation of the methyl signal δ 1·34 (26·7 ppm) enhanced signals at 1·52 (1·3%), 1·74 (4·4%), 1·90 (1·0%)

Table 4. ^{13}C and ^{1}H n.m.r. data for (3a)

Assignments are based on short-range (J 135 Hz, XHCORRD) and long-range (J 7 or 9 Hz, INAPT; J 10 Hz, COLOC) ^{13}C–^{1}H correlations

^{13}C (ppm)	^{1}H (δ)	^{13}C–^{1}H long-range correlations[A]	Carbon number
21·0 (q)	2·27 (t, J 1·8 Hz)	**115·8, 120·6, 137·3**	9-CH_3
25·7 (q)	1·41 (s)	**38·3, 72·2, 76·8**	2-CH_3
26·7 (q)	1·34 (s)	**32·4, 34·8, 38·3, 126·1**	6-CH_3
27·6 (t)	1·50 (m), *ax*		4
	1·52 (m), *eq*		
32·4 (s)			6
34·8 (t)	1·32 (m), *eq*		5
	1·74 (dt, J 5·9, 12·3 Hz), *ax*	**32·4, 126·1**	
38·3 (t)	1·45 (br d, J 13·2 Hz), *eq*		11
	1·90 (d, J 13·2 Hz), *ax*	**32·4, 126·1**	
72·2 (d)	3·69 (br s), *eq*[B]	**25·7, 34·8, 38·3, 76·8**	3
76·8 (s)			2
115·8 (d)	6·62 (s)		10
120·6 (d)	6·67 (dd, J 1·8, 7·8 Hz)	115·8	8
124·6 (d)	7·05 (d, J 7·8 Hz)	137·3, 154·6	7
126·1 (s)			6a
137·3 (s)			9
154·6 (s)			10a

[A] Assignments from INAPT (J 7 Hz) experiments are printed in **bold**; assignments from COLOC experiments are printed in light type.
[B] Assignments based on long-range (J 9 Hz, INAPT) ^{13}C–^{1}H correlations.

and 7·05 (4·1%). Irradiation of the methyl signal at δ 1·41 (25·7 ppm) enhanced the signals at 1·90 (1·4%) and 3·69 (2·1%). Irradiation at δ 1·74 (34·8 ppm) caused an enhancement of the signals 1·32 (6·5%) and 1·34 (1·1%). Irradiation at δ 1·90 caused enhancement of the signals 1·34 (2·2%), 1·41 (4·4%) and 1·45 (2·0%). Irradiation at δ 3·69 caused an enhancement of the signals at 1·41 (3·3%), 1·50 (1·3%) and 1·52 (4·4%). Compound (3) is therefore (2R*,3R*,6R*)-2,6,9-trimethyl-3,4,5,6-tetrahydro-2,6-methano-2H-1-benzoxocin-3-ol.

The genus *Laurencia* is known to produce at least 23 different sesquiterpenoid skeletons.[1] Of these, only the cuparane, laurane and cyclolaurane skeletal types consist of a non-fused bicyclic ring sytem which possesses an aromatic ring, but none contains metabolites similar to the cyclohexylphenol ring system isolated in this study. A survey of the relevant literature on marine natural products identified a number of substituted cyclohexylphenols as products of chemical syntheses (e.g.[7,8]) but none as natural products. It therefore appears that the isolated compounds constitute a regular isoprenoid sesquiterpene carbon skeleton not previous isolated as a natural product. Because of the novel nature of the carbon skeleton of these compounds, further chemical studies were carried out to confirm the structure of these molecules.

The presence of free hydroxy groups in (2) and (3a) was confirmed by acetylation. The presence of a methyl singlet at δ 2·32, the downfield shift of the aromatic protons, and no significant change in the shifts of the protons in the cyclohexane ring in the ^{1}H n.m.r. spectrum of the acetate of (2), were consistent with the

[7] Burnell, R. H., Jean, M., and Poirier, D., *Can. J. Chem.*, 1987, **65**, 775.
[8] Burnell, R. H., Jean, M., Poirier, D., and Savard, S., *Can. J. Chem.*, 1984, **62**, 2822.

fact that (2) was a phenol. Similarly, the presence of a methyl singlet at δ 2·10, a downfield shift of the proton signal at 3·69 to 4·85 in the saturated ring, and the unchanged shifts of the aromatic proton signals in the ^{1}H n.m.r. spectrum of (3b), the acetate of (3a), were consistent with the proposed structure of (3a) as a tricyclic compound containing an ether and a secondary hydroxy group. A tertiary alcohol function at C 2, with a secondary ether linkage at C 3, was thus ruled out as an alternative possibility for (3a).

To further investigate the relationship between the three compounds, attempts were made to convert (2) into (3a). Compound (2) underwent dehydrohalogenation when heated with potassium hydroxide in methanol under reflux to yield a single product (4).

The product (4) had the molecular formula $C_{15}H_{20}O$ as deduced by mass spectrometry and ^{13}C n.m.r. spectroscopy. High-resolution mass spectrometry could not be obtained on this semisynthetic compound. Compound (4) thus had six double-bond equivalents, and the presence of eight sp^2 carbons, six of which were in aromatic ring, dictated that the compound must be bicyclic as expected. The presence of signals in the ^{1}H n.m.r. spectrum for three aromatic protons at δ 6·50 (br s), 6·68 (dd, J 0·9, 7·6 Hz) and 6·96 (d, J 7·6 Hz), and an aromatic methyl group at 2·26 (s), confirms the presence of the 1,2,4-trisubstituted benzene ring. Similarly, the presence of a methyl signal at δ 1·74 (s) and a methine proton signal at 5·35 (br s) confirms the presence of unsaturation in the second ring.

A two-dimensional shift-correlated homonuclear n.m.r. experiment (COSYDQF) and one-dimensional (J 7 Hz, INAPT) and two-dimensional (J 10 Hz, XHCORRD; J 10 Hz, COLOC) heteronuclear correlation n.m.r. experiments allowed assignment of the proton and carbon signals in the molecule (Table 5). This established the position of the double bond and the vinyl methyl group in the cyclohexane ring.

The relative stereochemistry of the molecule (4) was established through n.O.e. experiments and proton coupling data (Table 5). Irradiation of the methyl signal at δ 1·37 (25·7 ppm) enhanced the signals at 2·05 (1·6%), 2·14 (1·4%) and 6·96 (1·1%). Irradiation of the methyl signal at δ 1·74 enhanced the signals at 2·14 (6·1%), 2·43 (3·3%) and 5·35 (3·9%). Irradiation of the methylene proton signal at δ 2·43 (42·4 ppm) enhanced the signals at 1·62 (9·1%), 1·74 (3·7%) and 2·14 (9·1%). Irradiation of the methine proton signal at δ 5·35 (120·6 ppm) caused an enhancement of the signal at 1·74 (5·3%). These data establish the relative stereochemistry of the molecule shown in the structure (4). Compound (4) is therefore (1′R*)-2-(1′,3′-dimethylcyclohex-3′-enyl)-5-methylphenol.

Epoxidation of (4) was carried out with *m*-chloroperbenzoic acid in the presence of sodium bicarbonate presumably to give an intermediate (5), which rearranged *in situ*. This overall reaction yielded a single product, the natural product (3a), this confirming the relationship between the two metabolites and the structure of (4).

Comparison of the ^{1}H n.m.r. spectra of the reisolated sesquiterpenes (6)–(10) with those of authentic compounds confirmed their identity in order of increasing polarity as follows: (6) (1S*,2S*,3S*,5S*,8S*,9S*)-tricyclo[6.3.0.0^{1,5}]undecan-2-ol,[9] (7)

[9] Coll, J. C., and Wright, A. D., *Aust. J. Chem.*, 1989, **42**, 1591.

isoobtusadiene,[9,10] (8) (1*Z*,8*R**,9*R**)-8-bromochamigra-1,11(12)-dien-9-ol,[2] (9) [1(15)*E*,2*Z*,4*S*,8*R*,9*S*]-8,15-dibromochamagra-1(15),2,11(12)-trien-9-ol[9] and (10) [1(15)*Z*,2*Z*,4*S*,8*R*,9*S*]-8,15-dibromochamigra-1(15),2,11(12)-trien-9-ol.[9,11,12]

Table 5. ^{13}C and ^{1}H n.m.r. data for (4)

Assignments are based on short-range (*J* 135 Hz, XHCORRD) and long-range (*J* 7 Hz, INAPT; *J* 10 Hz, COLOC) ^{13}C–^{1}H correlations

^{13}C (ppm)	^{1}H (δ)	^{13}C–^{1}H long-range correlations[A]	Carbon number
20·5 (q)	2·26 (s)	117·4, 121·0, 136·8	7
23·5 (t)	1·72 (m), *eq*		5′
	2·05 (m), *ax*		
23·7 (q)	1·74 (s)	**42·4, 120·6, 132·6**	8′
25·7 (q)	1·37 (s)	32·2, 36·7, 42·4, 130·9	7′
32·2 (t)	1·62 (m), *ax*		6′
	2·34 (m), *eq*		
36·7 (s)			2′
42·4 (t)	2·14 (br d, *J* 17·0 Hz), *eq*		6′
	2·43 (br d, *J* 17·0 Hz), *ax*		
117·4 (d)	6·50 (s)	121·0	6
120·6 (d)	5·35 (br s)	**23·5, 23·7, 32·2, 42·4**	4′
121·0 (d)	6·68 (dd, *J* 0·9, 7·7 Hz)		4
127·8 (d)	6·96 (d, *J* 7·7 Hz)	136·8, 154·0	3
130·9 (s)			2
132·6 (s)			3′
136·8 (s)			5
154·0 (s)			1

[A] Assignments from INAPT experiments are printed in **bold**; assignments from COLOC experiments are printed in light type.

The most polar compound (11) had similar ^{1}H and ^{13}C n.m.r. spectral data, and optical rotation and melting point to the previously reported compound isoobtusol.[13,14] Full characterization of the compound by n.m.r. was lacking in the literature. High-field two-dimensional n.m.r. and n.O.e. experiments were used to fully characterize the compound. This resulted in the reassignment of two carbon signals, the confirmation of four carbon signals previously deemed interchangeable, and the assignment of ^{1}H n.m.r. chemical shifts and coupling constants in addition to those previously reported.[14] Isoobtusol has been isolated from two species of *Laurencia, L. obtusa*[13–17] and *L. majuscula*.[3] The absolute configuration of isoobtusol has been determined by X-ray crystallogaphy[16] and

[10] Gerwick, W. H., Lopez, A., Davilla, R., and Albors, R., *J. Nat. Prod.*, 1987, **50**, 1131.
[11] Suzuki, M., and Kurosawa, E., *Tetrahedron Lett.*, 1978, **48**, 4805.
[12] Suzuki, M., Furusaki, A., Hasiba, N., and Kurosawa, E., *Tetrahedron Lett.*, 1979, **10**, 879.
[13] Gonzalez, A. G., Martin, J. D., Martin, V. S., and Norte, M., *Tetrahedron Lett.*, 1979b, **29**, 2719.
[14] Kennedy, D. J., Selby, I. A., and Thomson, R. H., *Phytochemistry*, 1988, **27**, 1761.
[15] Gonzalez, A. G., Darias, J., Diaz, A., Fourneron, J. D., Martin, J. D., and Perez, C., *Tetrahedron Lett.*, 1976, **35**, 3051.
[16] Gonzalez, A. G., Martin, J. D., Martin, V. S., Martinez-Ripoll, M., and Fayos, J., *Tetrahedron Lett.*, 1979a, **29**, 2717.
[17] Martin, J. D., Caballero, P., Fernandez, J. J., Norte, M., Perez, R., and Rodriguez, M. L., *Phytochemistry*, 1989, **28**, 3365.

has resulted in the reassignment of the original structure of the compounds.[15] Compound (11) had identical ^{13}C and ^{1}H n.m.r. spectral data to those previously reported (Table 6). The multiplicities of the carbon signals were established by a DEPT experiment. This showed the carbon signals 25·5 and 33·0 ppm to be CH_2 and CH_3, respectively, rather than CH_3 and CH_2 as previously reported.[14] The carbon shift at 33·0 ppm (CH_3) is consistent with similar compounds which bear an equatorial methyl group on a chlorinated carbon (cf. pacifenol[18]). A short-range two-dimensional heteronuclear experiment (J 135 Hz, XHCORRD), associated signals for all protons with the signals for the carbons to which they are attached (Table 6), while a two-dimensional proton homonuclear (COSY) experiment confirmed the previous assignments of proton resonances and coupling constants.[14] These experiments allowed the assignment of C 5 as 25·5, C 4 as 33·2 and C 1 as 33·7 ppm. Long-range correlations (J 7 Hz, INAPT) allowed the confirmation of the carbon assignments. The methyl proton signal at δ 1·90 (33·0 ppm) showed correlations to the carbon signals at 33·2, 65·0 and 71·1 ppm. This confirms the assignment of C 4 as 33·2 and C 14 as 33·0 ppm. The methyl proton signals at δ 1·04 (25·2 ppm) and 1·32 (24·8 ppm) showed connectivities to the carbon signals at 24·8, 25·2 and 43·5 ppm, which confirmed their assignments as either C 12 or C 13.

The assignment of C 12 and C 13 was resolved by an n.O.e. difference experiment. Irradiation of the methyl signal at δ 1·04 (25·2 ppm) caused an n.O.e. enhancement

Table 6. ^{13}C and ^{1}H n.m.r. data for isoobtusol (11)

Assignments are based on short-range (J 135 Hx, XHCORRD) and long-range (J 7 Hz, INAPT) ^{13}C–^{1}H correlations

^{13}C (ppm)	^{1}H (δ)	^{13}C–^{1}H long-range correlations	Carbon number
24·8[A] (q)	1·32 (s)	25·2, 43·5	12
25·2[A] (q)	1·04 (s)	24·8, 43·5	13
25·5[A] (t)	1·85 (dt, J 3·4, 3·4, 12·3 Hz)		5
	2·03 (td, J 3·4, 12·3, 12·3 Hz)		
33·0[A] (q)	1·90 (s)	33·2, 65·0, 71·1	14
33·2[A] (t)	1·80 (dt, J 3·4, 3·4, 12·3 Hz)		4
	2·21 (td, J 3·5, 12·3, 12·3 Hz)		
33·7 (t)	2·77 (dd, J 3·8, 15·7 Hz)		1
	3·06 (br d, J 15·7 Hz)		
39·2 (t)	2·41 (dd, J 3·9, 11·9 Hz)		8
	2·69 (t, J 11·9 Hz)		
43·5 (s)			11
43·7 (s)			6
65·0 (d)	4·42 (br s)		2
69·6 (d)	3·65 (br d)		9
71·1 (s)			3
76·2 (d)	4·40 (m)		10
113·8 (t)	4·90 (s)		15
	5·15 (s)	39·2, 147·4	
147·4 (s)			7

[A] Resonances which have been reassigned or confirmed.

[18] De Nys, R., Ph.D. Thesis, James Cook University of North Queensland, 1992.

of the methine signals at 4·40 (76·2 ppm, 3·9%) and 3·65 (69·6 ppm, 3·6%), the methylene signal at 1·85 (25·5 ppm, 2·8%) and the methyl signal at 1·32 (24·8 ppm, 1·4%). Irradiation of the methyl signal at δ 1·32 (24·8 ppm) caused an n.O.e. enhancement of the methine signal at 4·40 (76·2 ppm, 2·3%), the methylene signals at 2·77 (33·7 ppm, 3·0%) and 2·03 (25·5 ppm, 2·3%), and the methyl signal at 1·04 (25·2 ppm, 1·4%). The assignment of C 12 was therefore 24·8, and C 13, 25·2 ppm, which completed the characterization of isoobtusol.

The structure which corresponds to these data agrees with the X-ray crystallographic structure of Gonzalez *et al.*[16] However, it calls into question the structure of isoobtusol assigned by Kennedy *et al.*,[14] which appears to have the opposite stereochemistry at the spiro centre of the molecule, and has resulted in the misplacement of the chlorine in the cyclohexane ring.

Experimental

General

All solvents were distilled prior to use. Rapid chromatography[19] was carried out on silica gel 60G (Merck) and the elution of extracts was by stepwise gradient elution with mixtures of light petroleum and ethyl acetate. H.p.l.c. was carried out on a Waters Associates 6000A solvent delivery system connected to a Waters Associates model R401 differential refractometer. Infrared spectra were recorded on a Perkin–Elmer 1600 Fourier-transform infrared spectrometer as liquid films ($CHCl_3$), u.v. spectra were recorded in ethanol on a Varian series 634 spectrophotometer, while optical rotations were determined in chloroform solutions with a Perkin–Elmer 141 polarimeter. ^1H and ^{13}C n.m.r. spectra were recorded on a Bruker AM300 spectrometer. Low-resolution e.i. (70 eV) mass spectra were recorded in a VG707F mass spectrometer interfaced to a INCOS data system. High-resolution accurate mass measurements were carried out on the same instrument by using perfluorokerosene as the reference compound.

Collection, Extraction and Isolation

The sample was collected from Geoffrey Bay, Magnetic Island (28 September 1988) and was frozen and freeze dried. The freeze-dried tissue (270 g) was exhaustively extracted with dichloromethane and the resultant crude extract (10·2 g, 3·7%) was separated by rapid chromatography on silica gel to afforded 24 fractions.

H.p.l.c. separation (Techoprep 5-20 and Si-100 7 μm in tandem (ethyl acetate/light petroleum, 1:50) of the combined fractions 7, 8 and 9 yielded the sesquiterpenes (1), (2) and (3a) and the triquinane-based sesquiterpene (6).

(1′R,3′R*)-2-(3′-Chloro-1′,3′-dimethylcyclohexyl)-5-methylphenol* (1) (146 mg, 0·054%), mobile yellow oil, $[\alpha]_D$ +14° (*c*, 0·1) [Found: $M^{+\bullet}$ (e.i.m.s.), 252·1279. $C_{15}H_{21}{}^{35}ClO$ requires 252·1281]. λ_{max} (EtOH) 226 (ϵ 4081), 278 (2350), 284 nm (2265). ν_{max} (film) 3622, 2937, 2887, 2347, 1571, 1426, 1313, 860, 710 cm^{-1}. ^1H n.m.r. ($CDCl_3$, 300 MHz) see Table 1, (C_6D_6, 300 MHz) see Table 1. ^{13}C n.m.r. ($CDCl_3$, 75 MHz) see Table 1, (C_6D_6, 75 MHz) see Table 1. Mass spectrum *m/z* 254 (M+2, 5%), 252 (M, 18), 237 (24), 216 (12), 201 (43), 175 (33), 173 (67), 161 (41), 159 (19), 148 (52), 147 (11), 145 (18), 135 (30), 122 (10), 121 (100), 109 (17), 108 (49), 105 (24), 93 (23), 92 (32).

(1′R,3′R*,4′S*)-2-(4′-Chloro-1′,3′-dimethylcyclohexyl)-5-methylphenol* (2) (342·7 mg, 0·128%), brown mobile oil, $[\alpha]_D$ +47° (*c*, 0·1) [Found: $M^{+\bullet}$ (e.i.m.s.), 252·1275. $C_{15}H_{21}{}^{35}ClO$ requires 252·1281]. λ_{max} (EtOH) 222 (ϵ 4412), 277 (2177), 282 nm (2081). ν_{max} (film) 3603, 2954, 2872, 1617, 1297, 1188, 846, 710 cm^{-1}. ^1H n.m.r. ($CDCl_3$, 300 MHz) see Table 3. ^{13}C n.m.r. ($CDCl_3$, 75 MHz) see Table 3. Mass spectrum *m/z* 254 (M+2, 2%), 252 (M, 15), 237 (21), 201 (29), 173 (15), 161 (26), 159 (10), 148 (42), 145 (11), 135 (30), 133 (10), 122 (10), 121 (100), 109 (13), 108 (54).

[19] Coll, J. C., and Bowden, B. F., *J. Nat. Prod.*, 1986, **49**, 934.

Conversion of (2) into the Corresponding Acetate

Compound (2) (3·2 mg, 0·0127 mmol) was dissolved in dichloromethane (2 ml) to which was added triethylamine (1·1 mol. equiv., 1·5 mg) followed by dimethylaminopyridine (0·1 mol. equiv., 0·2 mg). Acetic anhydride (1·2 mol. equiv., 1·4 mg) was added to the reaction mixture. The reaction was complete (t.l.c.) after 4 h. On completion, the reaction mixture was diluted with water (2 ml) and washed successively with 1% hydrochloric acid (2 ml), 5% sodium bicarbonate (2 ml) and water (2 ml). The organic layer was separated and the solvents were removed under vacuum. The resultant product was purified by column chromatography (silica gel 60H, Merck; ethyl acetate/light petroleum, 1:10) to afford the acetate (3·0 mg, 95%).

(1′R,3′R*,4′S*)-2-(4′-Chloro-1′,3′-dimethylcyclohexyl)-5-methylphenyl acetate* (3·0 mg, 95%), brown mobile oil, $[\alpha]_D$ +22·3° (*c*, 0·3) [Found: $M^{+\bullet}$ (e.i.m.s.), 294·1386, $C_{17}H_{23}{}^{35}ClO_2$ requires 294·1387]. λ_{max} (EtOH) 220 nm (ϵ 4846). ν_{max} (film) 2927, 2854, 2361, 2341, 1745, 1465, 1376, 1236, 803 cm^{-1}. ^1H n.m.r. ($CDCl_3$, 300 MHz) δ 1·01, d, $(H8')^3$; 1·20, s, $(H7')_3$; 1·50–2·20, m, 7H; 2·30, s, $(H7)_3$; 2·32, s, CH_3; 4·22, br s, H4′; 6·79, s, H6; 6·99, br d, *J* 7·8 Hz, H4; 7·21, d, *J* 7·8 Hz, H3. Mass spectrum *m/z* 294 (M, <1%), 237 (14), 201 (14), 175 (12), 161 (15), 148 (18), 135 (12), 121 (37), 108 (24), 105 (13), 97 (18), 93 (11), 91 (16), 85 (28), 84 (11), 83 (28), 81 (14), 57 (100).

Elimination Reaction of (2) to Corresponding Alkene (4)

Compound (2) (20 mg) was dissolved in dry methanol (1 ml) to which was added potassium hydroxide (1 pellet) dissolved in dry methanol (1 ml). The reaction mixture was heated under reflux in a nitrogen atmosphere for 4 h (t.l.c.) at which time the reaction was quenched by addition of dilute hydrochloric acid (0·1 M); the mixture was subsequently washed with 4 aliquots of dichloromethane (5 ml) and the organic layer was separated. Solvents were removed under vacuum, and the reaction mixture was separated by column chromatography (silica gel 60H, Merck, ethyl acetate/light petroleum 1:10) to yield the alkene (4) (17 mg, 85%).

(1′*R**)-2-(1′,3′-dimethylcyclohex-3′-enyl)-5-methylphenol (4) (17 mg, 85%), yellow mobile oil, $[\alpha]_D$ +47·0° (*c*, 0·1). λ_{max} (EtOH) 276 (ϵ 8162), 219s (6270), 209 nm (3427). ν_{max} (film) 3606, 3186, 2924, 1710, 1620, 1503, 1412, 1156 cm^{-1}. ^1H n.m.r. ($CDCl_3$, 300 MHz) see Table 5. ^{13}C n.m.r. ($CDCl_3$, 75 MHz) see Table 5. Mass spectrum *m/z* 216 (M, 29%) 215 (26), 201 (17), 200 (15), 173 (60), 148 (78), 133 (25), 109 (21), 97 (38), 81 (49), 69 (83), 55 (100).

Epoxidation of (4)

The alkene (4) (24·3 mg) was dissolved in chloroform (2 ml) and cooled to 4°, and 1·2 mol. equiv. of *m*-chloroperbenzoic acid (25 mg) was added. The reaction was stirred at room temperature in the presence of sodium bicarbonate for 3 h. T.l.c. analysis of the reaction mixture at this time indicated that the reaction was complete. The reaction was quenched by the addition of water and the product was subsequently extracted with 3 aliquots of chloroform (5 ml). The organic layer was separated and the solvents were removed under vacuum. The resultant mixture was separated by h.p.l.c. (Techsil 5 silica, Si-100 7 μm in tandem; ethyl acetate/light petroleum, 1:8) to yield (3a) (20 mg, 82%) identical to the natural product.

(2R,3R*,6R*)-2,6,9-Trimethyl-3,4,5,6-tetrahydro-2,6-methano-2H-1-benzoxocin-3-ol* (3a) (20·9 mg, 0·007%), yellow viscous oil, $[\alpha]_D$ 0·00° (*c*, 0·1) [Found: $M^{+\bullet}$ (e.i.m.s.), 232·1463. $C_{15}H_{20}O_2$ requires 232·1463]. λ_{max} (EtOH) 220 (ϵ 4469), 277 (1957), 284 nm (1913). ν_{max} (film) 3627, 2928, 2358, 2336, 1504, 1146, 1056 cm^{-1}. ^1H n.m.r. ($CDCl_3$, 300 MHz) see Table 4. ^{13}C n.m.r. ($CDCl_3$, 75 MHz) see Table 4. Mass spectrum *m/z* 232 (M, 11%), 175 (13), 174 (14), 173 (100), 148 (20), 91 (12), 77 (12).

Conversion of (3a) into the Corresponding Acetate (3b)

Compound (3) (1·3 mg, 0·0056 mmol) was dissolved in dichloromethane (2 ml) to which was added triethylamine (1·1 mol. equiv., 0·7 mg) followed by dimethylaminopyridine (0·1 mol. equiv., 0·1 mg). Acetic anhydride (1·2 mol. equiv., 0·6 mg) was added to the reaction mixture. The reaction was complete (t.l.c.) after 4 h. On completion, the reaction mixture was diluted

with water (2 ml) and washed successively with 1% hydrochloric acid (2 ml), 5% sodium bicarbonate (2 ml) and water (2 ml). The organic layer was separated and the solvents were removed under vacuum. The resultant product was purified by column chromatography (Kieselgel 60H, Merck; ethyl acetate/light petroleum, 1:10) to afford 1·3 mg of (3b) (84%).

(2R,3R*,6R*)-2,6,9-Trimethyl-3,4,5,6-tetrahydro-2,6-methano-2H-1-benzoxocin-3-yl acetate* (3b) (1·3 mg, 100%), brown mobile oil, $[\alpha]_D$ +32·1° (*c*, 0·13) [Found: $M^{+\bullet}$ (e.i.m.s.), 274·1569. $C_{17}H_{22}O_3$ requires 274·1569]. λ_{max} (EtOH) 209 (ϵ 7826) 219 nm (6236). ν_{max} (film) 2926, 2854, 2361, 2341, 1745, 1465, 1376, 1237, 1226, 1046, 803 cm^{-1}. ^1H n.m.r. ($CDCl_3$, 300 MHz) δ 1·34, m, H5; 1·36, s, 6-CH_3; 1·43, s, 2-CH_3; 1·48, m, H11; 1·53, m, H4; 1·58, m, H4; 1·63, m, H5; 1·85, d, *J* 13·2 Hz, H11; 2·09, s, CH_3CO; 2·27, t, *J* 1·3 Hz, 9-CH_3; 4·85, br s, H3; 6·62, s, H10; 6·68, dd, *J* 1·8, 7·8 Hz, H8; 7·05, d, *J* 7·8 Hz, H7. Mass spectrum *m/z* 274 (M, <1%), 199 (10), 174 (12), 173 (89), 159 (11), 148 (19), 105 (15), 97 (16), 95 (11), 85 (22), 83 (26), 81 (15), 57 (100).

H.p.l.c. separation (Techoprep 5-20 and Si-100 7 μm in series; ethyl acetate/light petroleum, 1:10) of fraction 10 further yielded the sesquiterpene (1) and the triquinane (6) as well as the sesquiterpenes (7) and (9).

(1*S**,2*S**,3*S**,5*S**,8*S**,9*S**)-Tricyclo[6.3.0.0^{1,5}]undecan-2-ol (6) (51 mg, 0·019%), mobile oil, ^1H and ^{13}C n.m.r. data identical to those previously reported.[9]

Isoobtusadiene (7) (85 mg, 0·031%), clear mobile oil, ^1H and ^{13}C n.m.r. data identical to those previously published.[9,10]

[1(15)*E*,2*Z*,4*S*,8*R*,9*S*]-8,15-Dibromochamagra-1(15),2,11(12)-trien-9-ol (9) (60 mg, 0·022%), clear mobile oil, ^1H and ^{13}C n.m.r. data identical to those previously published.[9]

H.p.l.c. separation (Techoprep 5-20 and Si-100 7 μm in series; ethyl acetate/light petroleum, 1:10) of fraction 11 further yielded the sesquiterpenes (7) and (9) as well as the sesquiterpenes (8) and (10).

(1*Z*,8*R**,9*R**)-8-Bromochamigra-1,11(12)-dien-9-ol (8) (40 mg, 0·015%), clear mobile oil, ^1H and ^{13}C n.m.r. data identical to those previously published.[2]

[1(15)*Z*,2*Z*,4*S*,8*R*,9*S*]-8,15-Dibromochamigra-1(15),2,11(12)-trien-9-ol (10) (70 mg, 0·026%), clear mobile oil, ^1H and ^{13}C n.m.r. data identical to those previously published.[9,11,12]

H.p.l.c. separation (Techoprep 5-20 and Si-100 7 μm in series; ethyl acetate/light petroleum, 1:10) of fraction 13 further yielded the sesquiterpene (10) and the sesquiterpene (11).

Isoobtusol (11) (90 mg, 0·033%), colourless crystals, $[\alpha]_D$ and m.p. as previously reported.[13,16] λ_{max} (EtOH) 206 (ϵ 6006), 264 nm (3280). ν_{max} (film) 3556, 2957, 2932, 1636, 1448, 1371, 1244, 1069, 904 cm^{-1}. ^1H n.m.r. ($CDCl_3$, 300 MHz) see Table 6. ^{13}C n.m.r. ($CDCl_3$, 75 MHz) see Table 6.

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