Two-dimensional Nuclear Magnetic Resonance of Sesquiterpenes. Part 2.† Total Assignment of (+)-Aromadendrene, (+)-Longicyclene, and γ-Gurjunene by the Two-dimensional INADEQUATE Method

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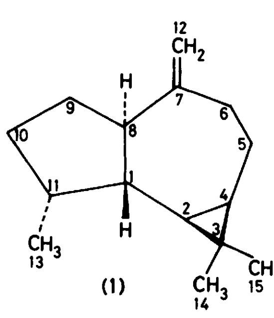
Autocorrelated ^{13}C–^{13}C double quantum coherence two-dimensional n.m.r. spectroscopy has been used for an unambiguous complete assignment of the ^{13}C resonances of three sesquiterpenes, (+)-aromadendrene, (+)-longicyclene, and γ-gurjunene

From an n.m.r. point of view, naturally occurring sesquiterpenes pose a number of problems for the assignment of ^1H and ^{13}C spectra. (+)-Aromadendrene (**1**) {(1*R*,2*S*,8*R*,11*R*)-7 methylene-3,3,11-trimethyltricyclo[6.3.0.0^{2,4}]undecane}, a constituent of numerous *Eucalyptus* oils, has recently been subjected to catalytic conversion[1] and used in the preparation of (+)-spathylenol.[2] (+)-Longicyclene (**2**) {(1*S*,2*S*)-1,2,6,6-tetramethyltricyclo[8.1.0.0^{2,8}0^{7,11}]undecane}, a constituent of the essential oil of *Pinus longifolia*,[3] has been obtained by photochemical transformation of longibornyl iodide[4] or by catalytic conversion of (+)-longifolene.[1] γ-Gurjunene (**3**) {(3*R*,6*R*,7*R*,10*R*)-6,10-dimethyl-3-isopropenylbicyclo[5.3.0]dec-1-ene} has been isolated from the gurjun balsams of several species of *Dipterocarpus*.[5]



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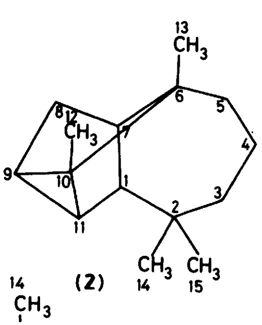

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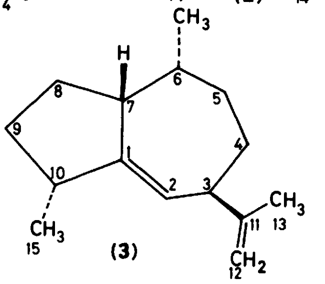

(3)

As these skeletons might be encountered in other natural derivatives, the present paper reports on the complete assignment of the n.m.r. spectra of these three sesquiterpenes using autocorrelated ^{13}C–^{13}C double quantum coherence two-dimensional n.m.r. spectroscopy.

Results and Discussion

The overall strategy chosen for the complete assignment of the ^{13}C n.m.r. spectra of sesquiterpenes (**1**)—(**3**) was first to

Table. ^{13}C N.m.r. chemical shifts of sesquiterpenes (1)—(3)

(+)-Aromadendrene (1)			(+)-Longicyclene (2)			γ-Gurjunene (3)		
δ[a]	Group[b]	Assignment[c]	δ[a]	Group[b]	Assignment[c]	δ[a]	Group[b]	Assignment[c]
154.00	C	7	58.64	CH	1	150.53	C	1
105.66	CH_2	12	46.32	C	6	146.81	C	11
53.80	CH	8	42.40	CH	7	122.26	CH	2
44.01	CH	1	41.83	CH_2	3	110.65	CH_2	12
39.17	CH_2	6	38.86	CH_2	5	47.85	CH	3
35.44	CH	11	33.91	CH_2	8	45.77	CH	7
35.23	CH_2	10	33.71	C	2	41.14	CH	10
29.53	CH_2	9	32.86	CH_3	15	34.05	CH	6
29.32	CH	2	28.86	CH_3	14	33.90	CH_2	9
28.74	CH_3	14	24.54	C	10	33.47	CH_2	5
27.71	CH	4	23.22	CH_3	13	30.06	CH_2	8
24.92	CH_2	5	23.12	CH	11	26.08	CH_2	4
19.94	C	3	20.96	CH_2	4	21.23	CH_3	13
17.15	CH_3	13	18.00	CH	9	19.69	CH_3	15
15.75	CH_3	15	10.53	CH_3	12	15.70	CH_3	14

[a] In p.p.m. from Me_4Si. [b] Determined from DEPT spectra. [c] Information obtained from double quantum coherence measurements.

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† Part 1, R. Faure, E. J. Vincent, E. M. Gaydou, and O. Rakotonirainy, *Magn. Reson. Chem.*, 1986, **24**, 883.

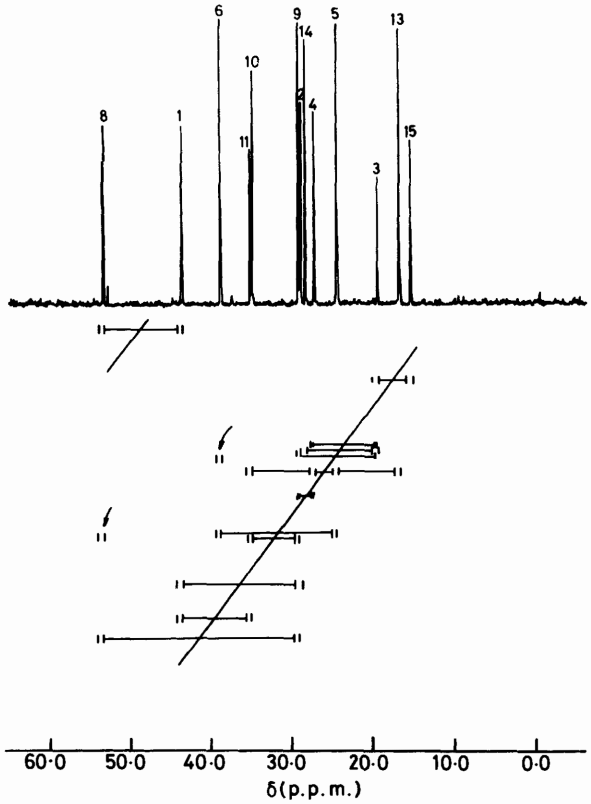

Figure 1. Two-dimensional ^{13}C two-dimensional INADEQUATE spectrum of (+)-aromadendrene (**1**). The connectivities are shown as horizontal lines connecting pairs of doublets distributed symmetrically with respect to the diagonal line of slope −2. Doublets marked with arrows are discussed in the text

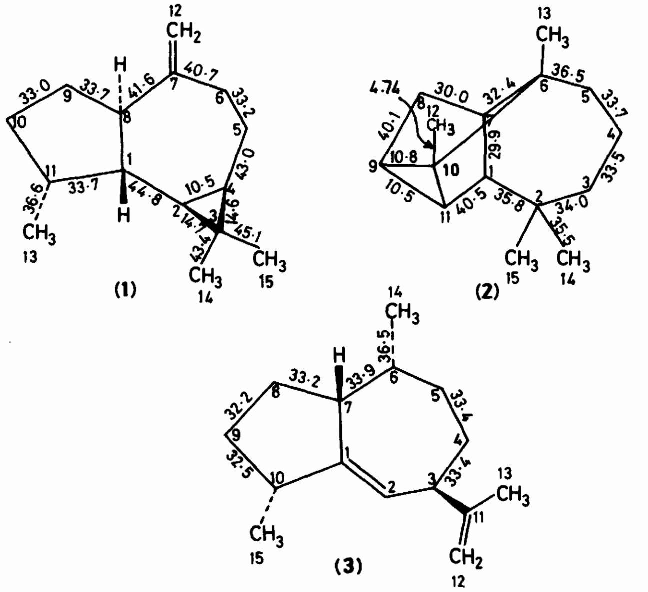

Figure 2. Natural-abundance one-bond ^{13}C–^{13}C coupling constants (in Hz) in sesquiterpenes (**1**)—(**3**) determined from double quantum coherence

ascertain the number of directly attached protons to each individual carbon, and then to establish the connectivity of the carbon skeleton of each molecule. The former was achieved from the DEPT pulse sequence,[6] while the later followed from the observation of one-bond carbon–carbon couplings in natural abundance by the 2D-INADEQUATE method.[7,8]

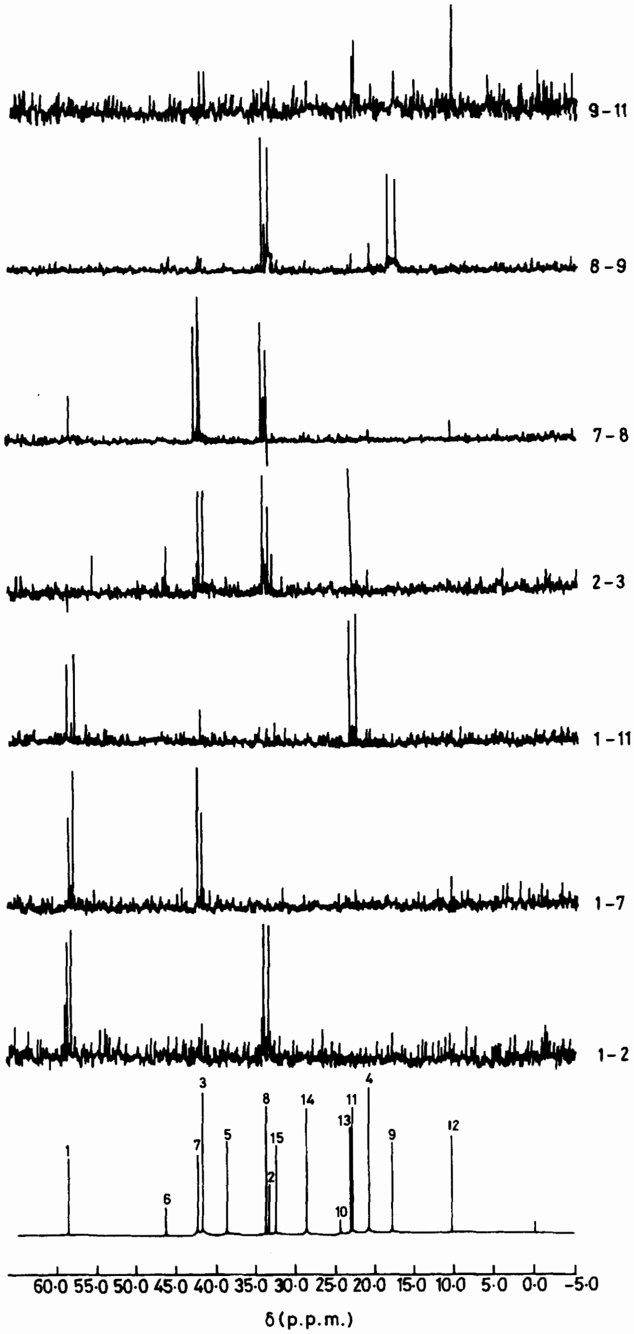

Figure 3. Examples of cross-sections parallel to the F_2 axis from ^{13}C two-dimensional INADEQUATE spectrum of (+)-longicyclene (**2**)

^{13}C *Spectral Assignments of* (+)-*Aromadendrene* (**1**).—Information about the multiplicity of each carbon was obtained from two DEPT experiments in which proton pulse values of 90 and 135° were employed. From these results (Table) quaternary and sp^2-hybridized carbons can be immediately assigned.

The autocorrelated two-dimensional double quantum coherence spectrum of the sp^3 region of (**1**) is shown in Figure 1. From this plot the carbon–carbon connectivities were unambiguously established as indicated by horizontal lines joining each pair of doublets which are centred about an axis of slope −2. The break in this axis is due to a wrong selection of

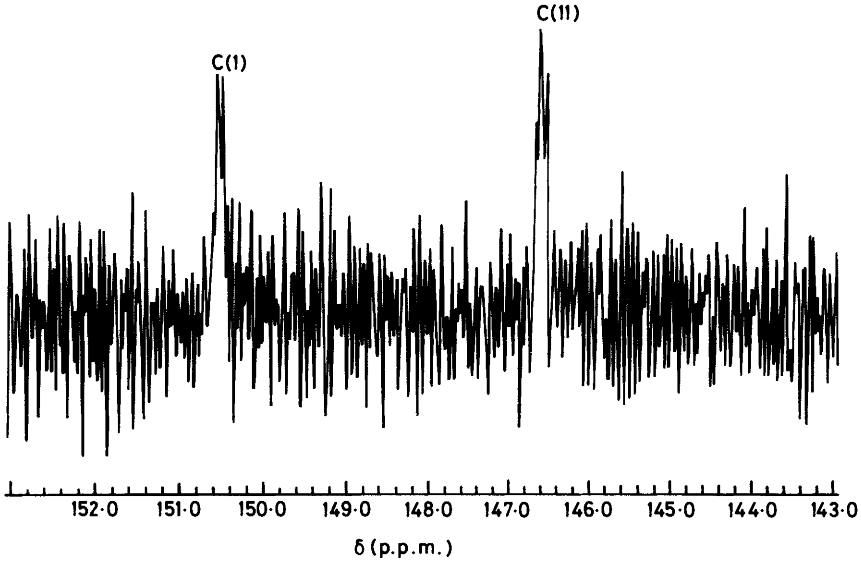

Figure 4. ^{13}C N.m.r. spectrum for C(1) and C(11) carbons of γ-gurjunene (**3**) with suppression of long-range couplings with aliphatic protons

the F_1 domain. However, folding in the double quantum dimension can be tolerated, since on the one hand there is no problem with filtering, and on the other hand it allows better digital resolution in this domain.[9]

The only unusual features concern the connectivities between C(7) and C(6) and C(8). Owing to the analogue filtering in the F_2 dimension which prevents folding in, only the high-field part of double quantum resonances was observed for the bonds C(7)–C(6) and C(7)–C(8). Moreover, since these doublets lie outside the double quantum spectral window, they appear folded in as shown in Figure 1 (signals marked with an arrow). Carbon-13 connectivities between cyclopropane carbons can be easily determined from the contour plot; each pair of doublets occurs with a coupling of *ca.* 10—15 Hz,[10,11] and, therefore, can be differentiated from the other double quantum resonances. A closer examination of the coupling constant values (Figure 2) reveals that ^1J(CC) is larger for carbons directly linked to the cyclopropane ring. This information could be used to aid the assignment of this kind of carbon in cyclopropane-containing sesquiterpenes.

Finally in the carbon–carbon connectivity plot the C(10)–C(11) bond is missing because it forms a strongly coupled AB system (Δδ *ca.* 0.2 p.p.m.). Such pairs of carbons require different conditions for optimal excitation *via* double quantum coherence.[12,13]

The complete ^{13}C chemical shifts assignment for (+)-aromadendrene is given in the Table, while one-bond carbon–carbon coupling constants are presented in Figure 2.

^{13}C *Spectral Assignment of* (+)*-Longicyclene* (**2**).—(+)-Longicyclene belongs to a class of molecules which is difficult to analyse by conventional n.m.r. spectroscopy because of the lack of multiple bonds or functional groups. Since a number of signals are within a very narrow chemical shift range, no assignments can be achieved from the spectral editing technique.

Plot of typical one-dimensional slices isolated from the two-dimensional INADEQUATE matrix is presented in Figure 3. From this spectrum the carbon–carbon connectivity is clearly demonstrated; moreover, since our experiment was optimized for the detection of sp^3–sp^3 carbon connections [$^1J(^{13}C–^{13}C)$ *ca.* 35 Hz], cyclopropane double quantum resonances have reduced intensity as shown in Figure 3.

Less than the total number of possible correlations was obtained from the two-dimensional INADEQUATE spectrum. Those missing are absent due to strong AB coupling [C(10)–C(11) and C(2)–C(15)] or to the low intensity of quaternary carbons [C(6)–C(10)]. These results lead to the chemical shift and coupling constant values compiled in the Table and Figure 2.

^{13}C *Spectral Assignment of* γ*-Gurjunene* (**3**).—Preliminary chemical shift information concerning γ-gurjunene was obtained from DEPT subspectra which provided the unambiguous assignment of sp^2-hybridized carbons C(2) and C(12). The multiplicity of each carbon is reported in the Table. Complete assignment of the aliphatic part of the ^{13}C spectrum of (**3**) was then achieved using the ^{13}C–^{13}C connectivity process.

In the two-dimensional INADEQUATE projection spectrum the ^{13}C coupling was missing for the methyl group located at δ 21.23 p.p.m.; this signal may thus be assigned as C(13). The starting point of the analysis is the methine carbon C(7) for which the resonance is unequivocally established at δ 45.77 p.p.m. *via* the double quantum spectrum: of the four CH signals only that at δ 45.77 p.p.m. shows couplings with methine and methylene signals but not with a methyl group. As a consequence, signals at δ 34.05 and 30.06 p.p.m. can be ascribed to C(6) and C(8), respectively. Of the two remaining methine carbons only the resonance at δ 41.14 p.p.m. exhibits a correlation with a methyl group; C(3), C(14), and C(15) are therefore assigned the values given in the Table. Final assignments of methylene carbons are straightforward based on the observation of correlated peaks and are given in the Table. In Figure 2 the C(6)–C(5) bond is missing due to strong coupling.

Finally, the assignment of sp^2-hybridized quaternary carbons C(1) and C(11) was achieved using a selective proton decoupling experiment. The irradiation frequency was set in the middle of the aliphatic region of the ^{1}H spectrum so that long-range J(CH) coupling between aliphatic protons and sp^2 carbons was suppressed and AX and AX_2 spectra were obtained for C(1) and C(11), respectively (Figure 4). It should be noted that the analysis of these carbons based on a comparison with model compounds such as monoterpenes[14] leads to a reversed assignment.

Conclusions.—Two-dimensional INADEQUATE experiments applied to compounds (**1**)—(**3**) allow the deduction of the structural characteristics of these sesquiterpenes. Further, they demonstrate the power and usefulness of this technique in routine applications to the structural elucidation of this kind of natural molecules, since this method reveals in a fully unambiguous way not only all the assignments but also the full sequence of carbons in the molecular structure.

Experimental

Materials.—(+)-Aromadendrene, (+)-longicyclene, and γ-gurjunene was purchased from Fluka.

Methods.—All n.m.r. experiments were carried out on a Bruker AM-200 spectrometer (Centre Interuniversitaire de RMN de Marseille). Samples for carbon-13 measurements were prepared in a 10 mm o.d. tube, by mixing sesquiterpene (0.8 ml) with $CDCl_3$ (1 ml). Tetramethylsilane was used as internal standard.

Resonance multiplicities were established *via* the acquisition of DEPT spectra obtained for proton pulse P_θ 90 (CH only) and 135° (CH and CH_3 differentiated from CH_2). For the DEPT sequence, the width of a ^{13}C 90° pulse was 13 μs, the width of a ^1H 90° pulse was 29 μs, and the $(2J)^{-1}$ delay was set equal to 3.7 ms.

The two-dimensional ^{13}C–^{13}C double quantum coherence spectra were acquired using spectral widths of 3 600—4 000 Hz, a 32-step phase cycling, and data acquisitions of 512 × 128 increments in t_1 to provide, after zero filling in the F_1 dimension, a matrix of 4 096 × 256 [$S(t_1,t_2)$]. Data files were processed using exponential broadening in both dimensions. The delay for the creation of the double quantum coherence was 7.1 ms, corresponding to coupling of 35.2 Hz, and the relaxation delay was 1—2 s. Total performance times for the experiments were *ca.* 35—40 h.

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