72.25; H, 11.95. **10** Calcd for $C_{14}H_{30}O_3$: C, 68.24; H, 12.27. Found: C, 68.49; H, 12.15. **11** Calcd for $C_{17}H_{32}O_2$: C, 76.06; H, 12.02. Found: C, 75.99; H, 11.86.

Nmr Spectra. Spectra of **1, 2, 3, 6a, 7,** and **8** are described in discussion section. **6b** (Klein *et al.*, 1964) δ 1.10 (s) overlapping with 1.05 (d) [12 *H* total], 1.6–2.3 (m, 4 *H*), 3.33 (s, 2 *H*), 3.6–3.9 (m, 2 *H*). **9** δ 1.12 (s, 3 *H*; t, 3 *H*), 1.40 (4 *H*), 1.72 (s, 3 *H*), 2.08 (m, 2 *H*), 3.32 (q, 2 *H*), 4.00 (d, 2 *H*), 5.40 (t, 1 *H*). **10** δ 1.08, 1.12 (t centered at 1.08, s at 1.08, s at 1.12; total 15 *H*), 1.38 (8 *H*), 3.1–3.75 (complex irregular multiplet > 8 peaks, 6 H). **11** δ 1.10 (s, 6 *H*; t, 3 *H*), 1.38 (4 *H*), 1.60 (s, 3 *H*), 1.68 (s, 3 *H*), 1.85–2.2 (m, 6 *H*), 3.30 (q, 2 *H*), 4.00 (d, 2 *H*), 5.07 (t, 1 *H*), 5.37 (t, 1 *H*).

Mass Spectra. Listed as follows *m/e* (% intensity of base peak): **1** *m/e* 170 (M^+, 40), 142 (13), 127 (20), 109 (11), 99 (100), 82 (100), 71 (100), 67 (100), 43 (100). **2** *m/e* 172 (M^+, 1.5), 157 (5), 129 (46), 127 (37), 111 (35), 109 (45), 81 (69), 43 (100). **3** *m/e* 170 (M^+, 8), 127 (52), 109 (54), 99 (27), 81 (100), 43 (91). **7** *m/e* 172 (M^+, <1), 139 (15), 136 (8), 128 (32), 127 (98), 109 (100), 95 (44), 81 (39), 71 (100), 69 (100), 43 (100), 41 (98). **8** *m/e* 154 (M^+, 4), 121 (9), 113 (98), 96 (23), 95 (100), 81 (72), 69 (98), 68 (100), 67 (87), 59 (94), 56 (94), 55 (51), 43 (100), 41 (100).

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Studies on Terpenes Using Carbon-13 Nuclear Magnetic Resonance Spectroscopy

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Methods of carbon-13 nmr spectroscopy have been reviewed. The spectral assignments for citronellol, citronellal, and related terpenes have been made using techniques of off-resonance decoupling, single frequency decoupling, and lanthanide-induced shift. An appreciable effect of the solvent on chemical shifts has been noted which must be taken into account for accurate measurement of chemical shift. For conformational analysis using cmr spectra, a simple additivity rule has been found to be useful but not entirely adequate. The potential of carbon-13 labeling for biosynthetic studies has been examined.

Carbon-13 nuclear magnetic resonance spectroscopy (cmr) was, until recently, the domain of physical chemists studying small molecules. The advent of spectrometers specially designed to overcome the poor sensitivity of ^{13}C nuclei and capable of averaging spectra over many hours of recording time attracted organic chemists interested in large and complex molecules (Weigert *et al.*, 1968). In the last 2 or 3 yr further advances in instrumentation have occurred; commercial units have become available for broad band proton decoupling (Ernst, 1966) and for rapid averaging of spectra by the Fourier Transform (FT) technique (Ernst and Anderson, 1966). Organic and bioorganic chemists are now flocking to this field. It is obvious that cmr spectroscopy has achieved a permanent place of importance among physical organic methods.

EXPERIMENTAL TECHNIQUES

For the present study, carbon-13 nmr spectra were recorded on a Bruker HX-90 spectrometer operating at 22.628 MHz and equipped with a Fourier Transform accessory consisting of a model BSV-2 pulse generator and power amplifier and a Fabri-Tek 1074 signal averaging device for accumulating the free induction decay signals. The Fourier transform was accomplished with a PDP-8L computer. Time for a 4K transformation required approximately 4.5 min. Broad band proton decoupling at 90 MHz was achieved with a Bruker BSV-2 power amplifier.

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INTERPRETATION OF CMR SPECTRA

The natural abundance of ^{13}C is sufficiently low (1.1%) so that ^{13}C–^{13}C spin–spin coupling can be neglected. Since protons have a spin of $^1/_2$, cmr signals are singlets, doublets, triplets, or quartets, depending on whether there are 0, 1, 2, or 3 hydrogens bonded to the carbon under observation. The coupling constants are usually large (100 to 200 Hz) so that the spectrum of a compound containing several carbon atoms may be quite complex due to overlap of signals. Long-range spin coupling with protons in the vicinity adds to the complexity of the spectrum. "Noise" or "broad-band" proton decoupling removes all the proton couplings and simplifies the cmr spectrum to a series of narrow singlets. Because of the Nuclear Overhauser Effect (NOE), which varies from one

Table I. cmr Chemical Shifts for Citronellol and Citronellal[a]

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Citronellol (I)	132.2	152.5	162.9	155.0	166.1	67.3	62.0	175.0	166.1	172.9
Citronellal (II)	−7.0	141.5	164.6	155.4	166.8	68.0	61.5	175.1	167.0	172.8

[a] In parts per million upfield from CS_2.

Table II. cmr Chemical Shifts of Nerol and Geraniol[a]

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Nerol (III)	134.0	66.9	55.1	160.2	165.4	68.0	61.1	175.0	175.6	169.2
Geraniol (IV)	133.8	67.8	55.1	152.7	165.6	68.0	61.6	166.9	166.9	175.0

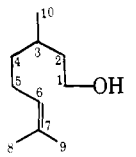
[a] In parts per million upfield from CS_2.

environment to another, the height of the signal is not necessarily directly proportional to the number of carbons producing the signal. This factor complicates the determination of the amount of carbon-13 incorporated in biological studies. For many compounds the number and type of carbons in a molecule can be determined by counting the number of peaks in the broad band proton decoupled spectrum.

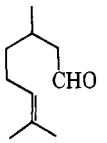
Several of the techniques for spectral interpretation that are currently in use are illustrated by considering the spectra of some terpenes we have studied.

SPECTRA OF CITRONELLOL AND CITRONELLAL

The broad band proton decoupled spectrum of citronellol (I) showed nine lines, indicating that two of the carbon atoms have the same chemical shift. The spectrum of citronellal (II) displayed individual peaks for each carbon. The carbinol



(I)



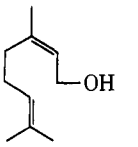
(II)

carbon of I, the aldehyde carbon of II, and the olefinic carbons of both terpenes were readily identified by their characteristic chemical shifts. Distinction between the two olefinic carbons could easily be made by single frequency off-resonance decoupling (SFOR). In SFOR decoupling the proton decoupling frequency is moved away from the proton resonances. This provides only partial decoupling while retaining some NOE enhancement in the carbon spectra. The carbons appear as perturbed quartets, triplets, or doublets, depending upon the number of attached protons. In this manner carbon-6 was differentiated from carbon-7 since the former became a doublet but the latter remained as a singlet on partial decoupling. The carbon-3 resonance also became a doublet on off-resonance decoupling and could be easily identified.

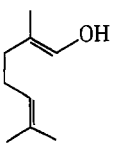
The technique of specific single frequency decoupling (Paul and Grant, 1964) was applied to the spectrum of citronellol. The cmr spectrum was observed while decoupling at relevant positions in the pmr spectra; sharpening of lines in the cmr spectrum indicated which carbon was coupled to specific protons. In the pmr spectrum of I the peaks corresponding to the methyl groups and the allylic protons at carbon-5 were easily identified; therefore the corresponding carbon resonances were readily located. It was noticed that carbon-5 and the carbon-9 methyl have the same chemical shifts. The carbon at carbon-3 was identified using the SFOR decoupling technique. This carbon showed a doublet under these conditions. The remaining resonance is assigned to carbon-4. The citronellal spectrum was assigned by analogy to citronellol. The assignments for both terpenes are shown in Table I.

CMR SPECTRA OF NEROL AND GERANIOL

The spectral assignment for nerol (III) and geraniol (IV) is shown in Table II. They were arrived at in a similar manner as that for citronellol and citronellal. Our results are in agreement with those reported previously (Jautelat *et al.*, 1970).

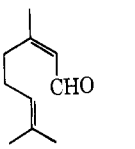


(III)

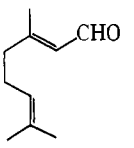


(IV)

The chemical shifts for carbon-5, carbon-6, carbon-7, carbon-8, and carbon-9 are very similar in the terpenes in Tables I and II. There is a small upfield shift of 0.9 ppm between the carbon-2 resonances of nerol and geraniol, which is apparently due to the difference in stereochemistry around the double bond. This steric effect (Grant and Cheney, 1967) is much more pronounced on the chemical shift of the allylic carbons. A comparison of the cmr spectra of neral (Z citral) (V) and geranial (E citral) (VI), reported by Jautelat *et al.* (1970), indicates that the difference in chemical shift of carbon-4 (and carbon-10) in these terpenes is of the order of 8 ppm, while there is little change in the chemical shift of carbon-1. The difference may be of advantage in conformational studies of these compounds.



(V)



(VI)

SHIFT REAGENTS AND CMR SPECTRA

A valuable technique for simplifying pmr spectra was developed by Hinckley (1969), who showed that large shifts are introduced for some peaks in the pmr spectrum of cholesterol upon the addition of a Europium complex. Subsequently various lanthanide complexes have been introduced as "shift reagents" for both upfield and downfield shifts. [For a recent review see Horrocks and Sipe (1971) and references cited therein; also see Wenkert *et al.* (1971).] The shifts have been assumed to be due to a pseudocontact interaction

Table III. Shift in cmr Spectra of Citronellol Induced by $Eu(Fod)_3$

Carbon	Sensitivity,[a] ppm
C-7	2.3
C-6	0.8
C-1	52.0
C-2	6.8
C-4	4.5
C-3	6.4
C-5	2.6
C-9	<0.8
C-10	4.5
C-8	0.8

[a] Sensitivity values represent the downfield shift (in ppm) which would be observed with a $Eu(Fod)_3$/substrate mole ratio of 1/1.

governed by the McConnell equation (McConnell and Robertson, 1958):

$$\frac{\Delta H}{H} = \frac{K(3\cos^2\theta - 1)}{r^3}$$

where K is a constant for a given molecule, r, the distance from the proton under observation to the lanthanide and θ, the angle of the same proton from the principal axis of the lanthanide–substrate complex. In general, the shift is proportional to the concentration of the shift reagent. It is convenient to extrapolate from a few observations the value of the shift for a proton for one equivalent of shift reagent and term it as "sensitivity" (see Table III).

As has been noted in an earlier section, the cmr spectrum of citronellol in CS_2 solution showed nine peaks, indicating that two carbons had the same chemical shift. In the hope of obtaining individual peaks for each carbon, pmr and cmr spectra of citronellol were recorded in carbon tetrachloride solution to which a small amount of the shift reagent Europium (III) [1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,5-octanedione ($Eu(Fod)_3$)] had been added. The cmr spectrum displayed noticeable shifts of certain peaks. In particular, the two carbon (carbon-5 and carbon-9) singlet was now resolved into two separate lines. As expected, the largest shift is in the resonance of carbon-1, the shift for other carbons is progressively smaller as the distance between them and carbon-1 increases (see Table III). Unexpectedly, the shift for carbon-7 is larger than that for the neighboring carbons.

SOLVENT EFFECTS AND REFERENCING

In order to determine the effect of solvents on the carbon-13 chemical shift a study was made on tetramethylsilane (TMS) which can serve as an internal reference for both pmr and cmr spectroscopy. In this study a 10-mm o.d. tube was used with a concentric 5-mm o.d. tube containing some TMS in hexafluorobenzene (C_6F_6) whose fluorine-19 resonance was used as a field frequency lock for the spectrometer. The outer annular region contained some TMS in the solvent under study.

Any observed chemical shift, δ_{obs}, in parts per million from TMS peak in C_6F_6 can be corrected for bulk diamagnetic susceptibility to give the corrected chemical shift, δ_{corr}, by using the equation:

$$\delta_{corr} = \delta_{obs} + \frac{2\pi}{3}(X_v \text{ ref} - X_v \text{sol})$$

in which X_v's are the bulk susceptibilities of the reference solvent (*i.e.*, C_6F_6) and the solvent under study. The solvent and C_6F_6 bulk susceptibilities have been employed because the small amount of added TMS will not significantly change their susceptibilities.

Table IV. Solvent Effects on the Carbon-13 Resonance of TMS[a]

Solvent	δc solvent[b]	$\Delta\delta$c TMS[c]
C_6H_6	128.67	1.51
CCl_4	99.53	3.56
CS_2	196.06	3.91
$CHCl_3$	80.98	2.91

[a] Corrected for bulk susceptibility differences. [b] Chemical shift difference of solvent line from external TMS. [c] Chemical shift difference between external and internal TMS.

Our observations are recorded in Table IV. It is obvious that there exists an appreciable solvent effect in cmr spectroscopy which has to be taken into account for accurate measurement of chemical shift.

CONFORMATIONAL ANALYSES BY CMR SPECTROSCOPY

In the past, pmr spectroscopy has provided a valuable insight on the conformation of various molecules, in particular cyclohexyl derivatives (Thomas, 1968, 1970; Booth, 1969). Additional information regarding conformation may be obtained by the cmr spectra of the same compounds. The chemical shifts of cyclohexyl carbons have been demonstrated to be sensitive to the conformation of substituents on the ring. Stothers and others (Buchanan and Stothers, 1969; Buchanan *et al.*, 1966, 1969) reported that the chemical shift of the carbinol carbon in cyclohexanol derivatives moves upfield by about 5 ppm when the hydroxyl group changes from the equatorial to the axial conformation. Anet *et al.* (1971) have observed the axial form of methylcyclohexane by cmr at low temperatures. Dalling and Grant (1967) have observed the cmr spectra of various methylcyclohexanes; Roberts *et al.* (1970) have studied cyclohexanols and cyclopentanols (Christl *et al.*, 1971). Variable temperature cmr spectra of substituted tetramethyl cyclohexanes have been studied (Doddrell *et al.*, 1970) as well as the spectra of dimethyl cyclohexanes and some decalins (Dalling *et al.*, 1971).

Additivity rules for predicting the carbon-13 chemical shift of carbons with various substituents have been developed (Malinowski *et al.*, 1966). The theoretical basis for such rules was subsequently presented (Vladimiroff and Malinowski, 1967). Additivity rules have also been formulated for substituted benzenes (Lauterbur, 1961), pyridines (Cotter, 1971), and indoles (Parker and Roberts, 1970).

We have examined the cmr spectra of variously substituted cyclohexanes (Dalling and Grant, 1967) and cyclohexanols (Roberts *et al.*, 1970) in the anticipation that an additivity rule can be derived that will include conformational factors also. The chemical shift of cyclohexane was used as a reference; divergence from this value is ascribed to the position and conformation of the substituent. By studying the cmr spectra of substituted cyclohexanes of known conformation, conformationally dependent substituent effects at the α, β, γ, and δ positions of a cyclohexane ring have been determined.

The *tert*-butyl group is generally accepted to be exclusively equatorial in cyclohexane derivatives. By comparing the cmr spectra of *tert*-butylcyclohexane and cyclohexane, the substituent parameters for the *tert*-Bu (equatorial) group were obtained. The substituent parameters are shown in Table V. The *tert*-butyl shift parameters can be used to determine the OH shift parameters for axial and equatorial OH groups from

Table V. Group Substituent Effects

Group	Conformation	Substituent parameter[a] C_α	C_β	C_γ	C_δ
t-Bu	Equatorial	−21.6	−0.9	−0.5	+0.1
Me	Equatorial	−5.7	−8.9	+0.0	+0.3
Me	Axial	−1.1	−5.2	+5.4	+0.2
OH	Equatorial	−43.2	−7.9	+2.5	+1.6
OH	Axial	−37.8	−5.5	+7.2	+0.7
Isopropyl	Equatorial	−17.4	−3.1	+2.8	−2.5

[a] In parts per million. Group substituent effects were determined using the data of Dalling and Grant (1967), Jautelat *et al.* (1970), and Roberts *et al.* (1970).

Table VI. cmr Spectra of Cyclohexanol

Shift[a]	C_α	C_β	C_γ	C_δ
Equatorial OH[b]	122.3	157.6	168.0	167.1
Axial OH[b]	127.7	160.0	172.7	166.2
Observed[c]	123.0	157.0	168.1	166.6

[a] In parts per million upfield from CS_2. [b] Calculated using the OH substituent parameters in Table IV. [c] Data of Roberts *et al.* (1970).

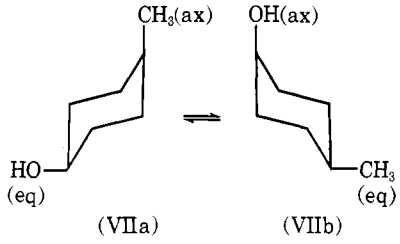
the spectra of *cis*- and *trans-tert*-butylcyclohexanol. The axial and equatorial substituent parameters determined for the OH group are given in Table V. Using the axial and equatorial OH shift parameters, the cmr spectra of cyclohexanol for both conformations are calculated (Table VI). From the results it appears that cyclohexanol exists predominantly in the conformation with the OH equatorial. Based on the carbon-1 chemical shift of cyclohexanol and the predicted shift for the axial and equatorial forms, the amount of each conformer can be determined.

$$\% \text{ equatorial} = \frac{\delta_{\text{carbon-1 ax}} - \delta_{\text{carbon-1 obs}}}{\delta_{\text{carbon-1 ax}} - \delta_{\text{carbon-1 eq}}} \times 100$$

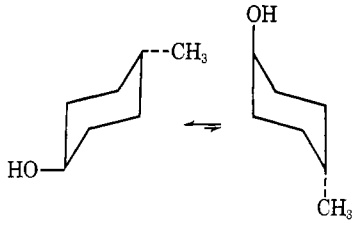
This gives 87% of the equatorial conformer and 13% of the axial conformer. The equilibrium constant for the process (ax $\rightleftarrows$ eq) is obtained from: K_{eq} = [equatorial]/[axial] = 6.7. The free energy difference is:

$$A = -\Delta F^\circ = RT \ln K = 1.22 \text{ kcal/mol}$$

The cmr spectra of methylcyclohexane and various methyl-substituted cyclohexanes have been obtained. The substituent parameters for the axial and equatorial methyl group (Dalling and Grant, 1967) are shown in Table IV. The methyl and OH substituent parameters can be used to calculate the cmr spectra of *cis*-4-methylcyclohexanol (VII) and *trans*-4-methylcyclohexanol (VIII). In *trans*-4-methylcyclohexanol one might assume that the diequatorial form (VIIIa) would be more favored than the diaxial form (VIIIb).



(VIIa) (VIIb)



(VIIIa) (VIIIb)

The calculations can be done for both forms and the best fit made to the observed spectra. Table VII shows the results together with the observed spectra. The fit of the data for the diequatorial form is almost exact. Similar calculations can be performed for *cis*-4-methylcyclohexanol. From the results in Table VII it appears tht *cis*-4-methylcyclohexanol has a much greater degree of conformational mobility than does *trans*-4-methylcyclohexanol. In the trans configuration both the OH and the methyl group can occupy the lower energy equatorial positions, thus favoring the diequatorial conformation over the diaxial conformation in which severe nonbonded interactions can occur. In the cis form, however, both conformations result in severe nonbonded interactions; hence, conformational mobility. The relative amounts of each conformer can be determined from the calculated shifts for carbon-1 and carbon-4 for both conformations and the observed shifts. These calculations show 74% of the axial OH conformer.

Based on the previous work, it seems desirable to apply the method to the determination of conformation in natural products. The menthols (IX–XII) provide closely related compounds which readily lend themselves to analysis. In addition to the methyl and hydroxyl shift parameters, the shift parameter for the isopropyl group in both axial and equatorial conformations is needed. Unfortunately this is not readily available for the axial conformer.

The equatorial isopropyl substituent parameter can be extracted from the carbon-13 chemical shifts of menthane determined by Jautelat *et al.* (1970). The equatorial isopropyl substituent parameters are shown in Table IV. The calculated and the observed carbon-13 chemical shifts for menthol, isomenthol, and neomenthol are shown in Table VIII. The chemical shift of the carbon-10 methyl group is least affected by the conformation of substituents at carbon-3 and carbon-4 in the ring; it is, however, sensitive to the conformation of the ring as it changes the methyl group from the axial to the equatorial conformation. The discrepancy between the carbon-10 chemical shift for isomenthol and the calculated value for an axial carbon-10 methyl group indicates considerable conformational mobility for isomenthol. Work in progress, including variable temperature cmr spectra on the menthols, should allow preferred conformations to be determined.

On the basis of the data reported here it appears that the

Table VII. cmr Spectra of *cis*- and *trans*-4-Methylcyclohexanol

Shift[a]	C-1	C-2	C-3	C-4
***trans*-4-methylcyclohexanol**				
(VIIIa) equatorial OH, equatorial Me[b]	122.6	157.6	159.1	164.4
(VIIIb) axial OH, axial Me[b]	127.9	165.4	165.2	167.5
Observed	122.8	157.7	159.4	161.1
***cis*-4-methylcyclohexanol**				
(VIIa) equatorial OH, axial Me[b]	122.5	163.0	162.8	166.0
(VIIb) axial OH, equatorial Me[b]	128.0	160.0	164.0	160.5
Observed[c]	126.6	161.1	163.8	161.9

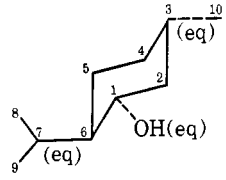
[a] In parts per million upfield from CS_2. [b] Calculated using the OH and Me substituent parameters in Table IV. [c] Data of Roberts *et al.* (1970).

Table VIII. cmr Spectra of Menthol, Neomenthol, and Isomenthol[a]

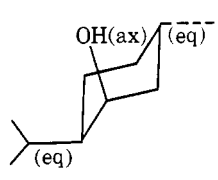
	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
(IX) Menthol obs	121.2	146.8	157.4	160.5	168.6	141.9	166.3	171.2	175.8	169.9
calcd[b]	119.2	151.5	159.8	161.0	164.9	140.5	166.7	171.5	176.5	169.7
(X) Neomenthol obs	125.1	144.1	168.1	156.9	163.0	149.1	166.5	171.1	171.6	169.8
calcd[b]	124.6	142.9	169.6	160.1	164.4	153.9	166.7	171.5	176.5	169.7
(XIb) Isomenthol obs	124.8	152.6	159.7	165.4	171.9	142.9	166.6	171.9	174.7	172.9
calcd[b]	124.7	155.2	164.7	164.7	170.3	140.4	166.7	171.5	176.5	173.7

[a] In parts per million upfield from CS_2. [b] Calculated for the conformation with the isopropyl group equatorial.

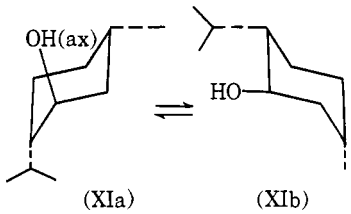
additivity rule is a promising approach to the conformational study of cyclohexane derivatives but more work will have to be done to determine the adequacy of this simple additivity relationship.



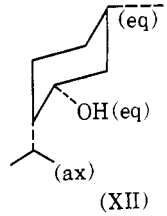
(IX)
Menthol



(X)
Neomenthol



(XIa) (XIb)
Isomenthol



(XII)
Neoisomenthol

BIOSYNTHETIC STUDIES

The availability of instrumentation for recording proton decoupled cmr spectra of compounds in solution has significant implications for studies on biosynthesis and on drug metabolism. With the current models of spectrometers it is possible to determine in a qualitative fashion (and in some cases in a semiquantitative fashion) the extent and site of incorporation of ^{13}C in a molecule by a direct comparison of the spectra of labeled and unlabeled compounds (Tanabe *et al.*, 1970).

As an illustration of the power of cmr spectroscopy as a tool for biosynthetic studies, we can cite the recent investigation (Neuss *et al.*, 1971) on cephalosporin C which contains 16 carbon atoms and various functional groups (Figure 1).

A considerable amount of effort was necessary for establishing the spectral assignments by off-resonance decoupling, single frequency decoupling, and the comparison of the cmr spectra of closely related compounds. Once the chemical shifts of the various carbons had been determined, the stage was set for rapid evaluation of incorporation of ^{13}C-labeled substrates. The integrated intensity data for labeled and unlabeled cephalosporin were obtained from spectra recorded on the same day with the same instrument settings using samples of identical concentration.

In one experiment cephalosporin C was labeled with [1-^{13}C]sodium acetate and in another with [2-^{13}C]sodium acetate. The isotope level was 62–63% of ^{13}C in these substrates. Relative intensities of various peaks in labeled and unlabeled cephalosporin C indicated that certain carbon atoms had been enriched to the level of 2–5% ^{13}C (error ± 20%).

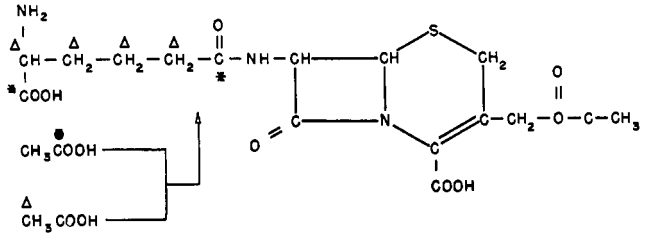


Figure 1. Labeling pattern for cephalosporin C

The labeling pattern at multiple sites was thus established just from one spectral measurement. This is in contrast with the multistep bond-specific chemical degradation and scrupulous purification at various steps that would have been required for obtaining the same information using ^{14}C-labeled substrates.

Shift reagents can be particularly useful in examining complex spectra of compounds containing various functional groups that coordinate with lanthanides. They can ensure that each carbon in the cmr spectrum will be resolved from the other carbons in the molecule and thus be amenable to accurate intensity measurements. The recent availability of ^{13}C-labeled compounds with 90% isotope abundance at a much lower price than before has greatly increased the utility of ^{13}C-labeled precursors for biosynthetic studies. Work along these lines is in progress in our laboratory for studying the role of amino acids in the biosynthetic of isoprenoids.

In less than 15 yr pmr spectroscopy has undergone tremendous development and sophistication. In the light of this experience it is safe to predict that newer techniques and newer instrumentation (see for example, Gansow and Schittenhelm, 1971) will appear that will make cmr spectroscopy an indispensible tool in organic and bioorganic laboratories.

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Olfactory Studies on Enantiomeric Eremophilane Sesquiterpenoids

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Odor threshold concentrations, determined in the liquid phase as well as in the vapor phase, are reported for five enantiomeric pairs of sesquiterpene ketones, including (+)- and (−)-nootkatone and (+)- and (−)-α-vetivone. For these last two pairs comparative taste experiments were also carried out. Odor profiles for all compounds are presented. Significant differences, both in odor strength and in odor character, have been found between all enantiomeric pairs and are particularly large for the nootkatones. (−)-Nootkatone has only a weak odor and lacks the characteristic grapefruit flavor of its dextrorotatory enantiomer.

In 1969 Naves reviewed the work done on enantiomeric forms of odorous substances and concluded that enantiomers can differ in odor quality as well as in odor strength. This conclusion, at that time perhaps a little premature, and questioned by Wright (1964), has since then been corroborated by some excellent investigations. Among these may be mentioned those on linalool by Ohloff and Klein (1962), on carvone by Russell and Hills (1969) and by Friedman and Miller (1971), and on derivatives of α- and β-pinene by Theimer and McDaniel (1971).

At present it seems generally accepted that enantiomers can differ in odor. Since this fact may well become of principal importance for the elucidation of the mechanism of olfaction, additional data may be useful. The present work was aimed at obtaining more information on the subject by studying the olfactive properties of the pure enantiomeric forms of nootkatone, α-vetivone, and some related compounds. The results are compared with recent work on (+)-nootkatone and some derivatives by Stevens *et al.* (1970).

EXPERIMENTAL

Synthesis. The synthesis of optically active nootkatone had thus far only been possible from the closely related sesquiterpenes (+)-valencene (Hunter and Brogden, 1965) and (+)-nootkatone (Erdtman and Topliss, 1957; Firmenich, 1969). Recently a stereoselective synthesis leading to optically active sesquiterpenoid ketones of the eremophilane series was developed in our laboratories (Gen *et al.*, 1971). The levorotatory form of a tricyclic ketone, here referred to as "tricycloketone" (Figure 1), was prepared from (−)-sabinene and converted to (+)-8-dehydro-11,12-dihydronootkatone and (+)-nootkatone. This nootkatone was identical with the (+)-nootkatone prepared from (+)-valencene (Hunter and Brogden, 1965). (+)-α-Vetivone was obtained by acid-catalyzed isomerization of (+)-nootkatone. (+)-Tetrahydronootkatone was prepared by hydrogenation of (+)-nootkatone according to Erdtman and Hirose (1962). By starting the reaction sequence with (+)-sabinene the opposite enantiomeric forms of the compounds mentioned were obtained. Figure 1 shows the structural formulas of both series of compounds. Some physical constants are presented in Table I.

PURIFICATION. Since very small amounts of impurities can have a large influence on the odor, the utmost attention was paid to the purification of the compounds. Before being used in further synthetic steps, the key compounds, the tricycloketones, were repeatedly crystallized until the enantiomers had identical optical rotations of opposite sign ($\alpha^{20}D$ = +216° or −216°). The other crystalline materials, nootkatone and α-vetivone, were likewise purified by repeated crystallization (five times or more). Shortly before the sensory tests these compounds were crystallized twice. The liquid tetrahydro- and dehydrodihydronootkatones were

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