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The Mass Spectra of [17-^{13}C]Phyllocladene and [3-^{13}C]Methylenecholestane—III†

R. A. Cruz, R. C. Castillo and F. J. Garcia‡
Instituto de Química, Piso 11, Torre de Ciencias, México 20, D. F. México
R. I. Reed
University of Glasgow, Glasgow G 128 QQ, Scotland

The mass spectra of [17-^{13}C]phyllocladene and [3-^{13}C]methylenecholestane have been examined. It is shown that there are some rearrangements at 70 eV as in the case of [17-^{13}C]kaurene. However, no extensive randomization is evident at the molecular ion level. The results are interesting because very little is known about ^{13}C randomization in polycyclic aliphatic hydrocarbons. The percentage retention of label was calculated for each ion.

INTRODUCTION

In spite of the great theoretical interest of ^{13}C labelling of organic compounds in mass spectrometry, there is very little experimental work reported in this area. In 1970 several important examples of extensive randomization ('scrambling') were published for benzene by Horman *et al.*[1] and Perry *et al.*,[2] for toulene by Siegel;[3] for benzothiophene by Cooks and Bernaseck,[4] and for butyl iodide by Davis.[5] More recently Lin and Harrison[6] reported similar results for isobutene, and have proposed a multistep mechanism by way of explanation. The questions arise as to whether extensive randomization ('scrambling') is a general phenomenon and what happens with large molecules. The information about ^{13}C randomization in polycyclic aliphatic hydrocarbons, for example, is very scarce. In order to answer these questions, we started work on ^{13}C labelled compounds in 1972.[7] In a continuation of this work, we now report the study of the mass spectra of [17-^{13}C]phyllocladene and [3-^{13}C]methylenecholestane. The former is a stereoisomer of kaurene, whose mass spectrum was reported earlier.[8]

EXPERIMENTAL

Spectra were obtained using an Hitachi-Perkin-Elmer RMU-7H double focusing instrument. Samples were introduced through a heated glass inlet system at 150 °C. The ionizing current was held at 80 μA and the ionizing voltage at 70 eV. Low rate scans (100 s per decade) were run for both the ^{13}C enriched and the normal compounds. The percentage of retention was calculated from the relative peak intensities with the aid of a computer program which is described in the Appendix. The ^{13}C labelled compounds were synthesized from the corresponding norketones by means of the Wittig reaction, employing a technique described previously.[7] The labelled methyl iodide (from Bio-Rad) was 62.4% ^{13}C enriched, as determined by mass spectrometry. The standard deviations of the retention of label for peaks of more than 10% relative intensities are between ±3 and ±2%, while for those with less than 10% relative intensities they are between ±5 and ±6%.

† Part II, 'The Mass Spectra of Carbon-13 Labelled Kaurene and some Related Compounds'; Ref. 7.
‡ To whom correspondence should be addressed.

RESULTS

The mass spectra of these two unsaturated labelled and unlabelled hydrocarbons have been compared employing the computer program described in the Appendix. The retention of label in each of the peaks of the spectra has been determined. The appropriate values for kaurene and phyllocladene are reported in Table 1, and those for methylene cholestane in Table 2.

Table 1. Retention of ^{13}C label at each *m/e* value

		Kaurene		Phyllocladene	
Assignment[a]	*m/e*	% Rel. int.	% Label	% Rel. int.	% Label
$[M-15]^+$	257	40	95	17	95
$[M-28]^+$	244	5	95	3	62
	243	4	86	3	100
	230	29	80	8	0
a_1	229	85	80	22	61
	215	8	100	3	95
	201	11	75	3	73
a_2	187	39	93	10	100
	173	10	61	6	68
	159	19	71	10	100
b_2	147	42	82	13	80
b_3	137	35	8	11	37
b_4	133	32	59	16	92
b_1	123	62	12	27	21
b_5	121	27	70	12	62
	119	41	77	22	52
	109	46	18	26	40

[a] See Scheme 1.

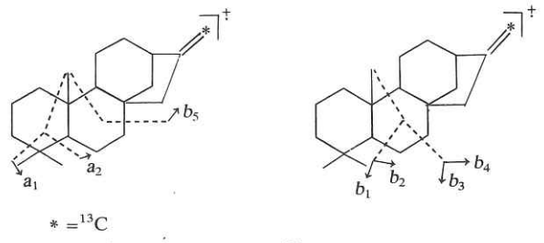
Table 2. Retention of ^{13}C label at each *m/e* value

Assignment[a]	[3-^{13}C]Methylenecholestane *m/e*	% Rel. int.	% Label
$[M-15]^+$	369	21	94
a_2	328	23	0
a_1	316	62	14
a_1	315	13	0
r_2	299	3	100
	287	5	0
b_2	275	6	2
r_1	271	14	93
b_4	262	3	23
b_4	260	2	0
b_6	248	2	0
d_2	244	14	100
d_1	229	40	90
c_3	190	6	63
c_2	174	10	100
c_1	162	22	22
b_5	136	11	94
b_3	122	46	100
b_1	107	75	73

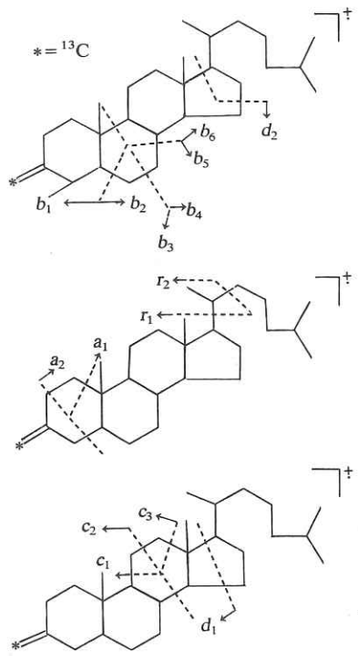
[a] See Scheme 2.

The corresponding peak assignments, indicating the probable origin for each peak, are shown in Schemes 1 and 2. As has been observed previously,[9] there are hydrogen transfers usually involving loss of hydrogen from the charged fragment; phyllocladene and kaurene show similar trends in the relative retention or elimination of label, although the quantitative values are often markedly different. Two notable differences appear for the ions $[M-28]^+$ and $[M-43]^+$, where the loss of label must be accompanied by a rearrangement. It is possible that the first step in this rearrangement is a double bond migration, and that this bond migration would be more favourable for phyllocladene due to steric reasons. The more significant fragmentations observed for phyllocladene, although not fully specific, are in good agreement with those expected from the empirical rules in organic mass spectrometry, and can be predicted in terms of the statistical approach as pointed out previously.[8] The main fragmentations appear to occur across rings A and B.

The results for [3-^{13}C]methylenecholestane show that there is rather specific retention or elimination of label, and that this occurs even at very close *m/e* ratios, for example at *m/e* 123 and 121 (Table 2). This again shows that there is no extensive randomization of label at the molecular ion level. These results indicate that, in spite of possible rearrangements, the usual rules for hydrocarbon fragmentation with due allowance for all statistically favourable bond ruptures,



Scheme 1. Fragmentation of [17-^{13}C]phyllocladene.



Scheme 2. Fragmentation of [3-^{13}C]methylenecholestane.

still apply. In some cases peaks were found for which retention or elimination of label was not specific, so that the same fragment could arise from several molecular precursors.[9] Specially favourable fragmentations include the scission of the sidechain (ring D) and the well known ruptures across ring D.[10] There are also two fragmentations across ring A which are apparently induced by allylic bond ruptures.

DISCUSSION

The presence of at least partial retention or elimination of label in the fragmentation of these compounds shows that extensive randomization is absent at the molecular ion level. Extensive randomization of the structural isomers of phyllocladene and kaurene would lead to the same fragmentation patterns, and also equal retention of label. Partial randomization is nevertheless present, and this can be accounted for by some rearrangements, and by a multiplicity of origins for most fragments. However, there are certain main fragmentation paths which in general are in good agreement with the empirical fragmentation rules in mass spectrometry.[11]

The fact that extensive randomization is absent is in sharp contrast to the behaviour of propene and butene, but this is reasonable because there must be orbital symmetry and distance restrictions for a multistep mechanism of randomization. (Recently a distinction has been made between scrambling and randomization,[12] with the recommendation being made to abandon the use of the term 'scrambling' in order to describe H-D randomizing processes. We feel that this recommendation should be extended to encompass ^{13}C labelled compounds.)

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APPENDIX

A computer program† was designed in order to calculate automatically the percentage of retention of label at each *m/e* ratio along the following lines:

(1) Normalization was carried out by groups‡ to minimize the effects of change in sample pressure. Each group in the unlabelled spectra was compared with the corresponding group in the labelled spectra according to the formulae

$$f(j)\sum_{i}^{\text{Group } j} I_{ME}(i) = \sum_{i}^{\text{Group } j} I_{SM}(i)$$

$$f(j) = \frac{\sum_{i}^{\text{Group } j} I_{SM}(i)}{\sum_{i}^{\text{Group } j} I_{ME}(i)}$$

where: I_{SM} = intensity for the unlabelled spectra at $m/e = i$ in group j and I_{ME} = intensity for the labelled spectra at $m/e = i$ in group j.

The sums run over all peaks of j group

$f(j)$ = normalization factor for group j.

† The full program is available on request.
‡ The groups are collections of peaks sometimes arbitrarily defined.

(2) The artificial spectra of the completely labelled compounds were obtained according to

$$I_M(i) = I_{ME}(i) - FI_{SM}(i) \text{ (for each } i)$$

where F is obtained from the relative intensities at the molecular ion as

$$F = \frac{I_{ME}(M)}{I_{SM}(M)}$$

$M = m/e$ of molecular ion of the unlabelled compound.

(3) Calculation of the % of label $Z_{i,i+1}$ = intensity transferred from $m/e = i$ to $m/e = i+1$ due to labelling.

Then the corrected intensity of each peak in the labelled spectra will be

$$I_M(i) = I_{SM}(i) + Z_{i-1,i} - Z_{i,i+1}$$

where

$$Z_{i,i+1} = \sum_{k}^{i} [I_{SM}(k) - I_M(k)]$$

and

$k = m/e$ initial for the group

$$\% \text{ of retention} = \frac{Z_{i,i+1}}{I_{SM}(i)} \times 100$$