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The Mass Spectra of Oxygen Heterocycles I.
The 4-Hydroxy-3-phenylcoumarins (*iso*Flavonols).

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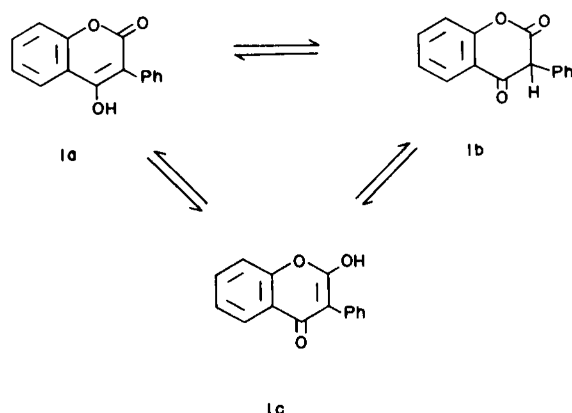
The unique breakdown patterns of the 4-hydroxy-3-phenylcoumarins (isoflavonols) in the mass spectrometer have been examined. Explanations of the fragmentations found have been confirmed by deuteration studies and by an examination of the mass spectra of 4-methoxy-3-phenylcoumarins. The scheme has been extended to 4,7-dihydroxycoumarin, which is contrasted with 4-methyl-7-hydroxycoumarin.

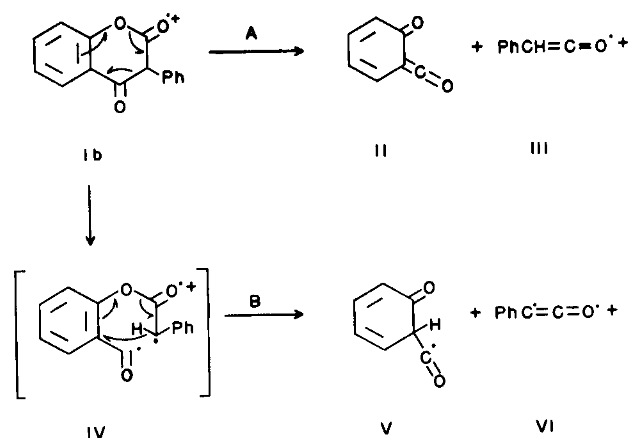
We have recently been engaged in the characterisation of a new type of natural product, the 4-hydroxy-3-phenylcoumarins (1,2,3) or *iso*flavonols, and amongst other properties we have investigated their behaviour when subjected to electron bombardment. Although these compounds are isomers of the flavonols, it was at once obvious that the breakdown pattern of the two classes of compound was totally different. Moreover the behaviour of the isoflavonols was separate from that of any other flavonoid in which the heterocyclic ring is totally unsaturated.

As the results were promising from the viewpoint of structure determination, as well as from intrinsic interest, a fuller study of simple *iso*flavonols was made. These compounds may exist in any one of three forms (Ia-c), all of which are interconvertible by simple prototropy. We have shown that it is the existence of form (Ib) that allows breakdown in the mass spectrometer. In this form the *iso*flavonols



are more akin to the reduced flavonoids, such as flavanones, rather than to the flavones or flavonols. Although flavone itself breaks down mainly by a reverse Diels-Alder reaction (4), flavones substituted with a hydroxyl or methoxyl groups exhibit this behaviour only to a minor degree (5,6). Presumably the unsaturation in ring C allows stabilisation of the molecular ion by mesomerism over the whole molecule, behaviour not possible to the reduced flavonoids. The striking and predictable contrast between the mass spectra of 4,7-dihydroxycoumarin and 4-methyl-7-hydroxycoumarin illustrates the importance of forms analogous to (Ib) in this series also.

We have been able to define three general modes of breakdown of the *iso*flavonols in the mass spectrometer. The two most predominant, hereafter called path A and path B, are shown below. In path A a

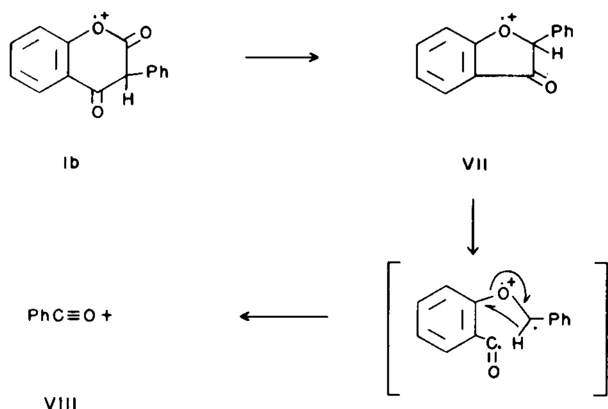


direct reverse Diels-Alder reaction proceeds to give fragments (II) and (III). With path B, fragmentation is preceeded by formation of the charged radical (IV). Cleavage of the C_3-C_4 bond is facilitated by the fact that the free electron at both positions may be stabilised by resonance with the adjacent benzene ring. A hydrogen atom transfer then occurs as shown to yield fragments (V) and (VI).

In each case, of course, if the electron initially removed is associated with the oxygen attached to C_4 rather than C_2, then fragments (II) and (V) will

bear the charge. All the fragments are capable of stabilising the charge and in fact, spectra will be presented in which both types of fragment are seen. The tendency is for fragments containing methoxyl groups to be more stabilised than those containing hydroxyl groups.

A third, and in general, minor mode of breakdown will be called path C. In this pathway, extrusion of carbon monoxide occurs, followed by the



breakdowns shown to give fragments (VII) and (VIII).

At this stage it may be worthwhile to state that whereas these processes have been shown to occur, the actual state of the fragments is not known. Thus some may be tropolone type cations rather than the form written. Lacking evidence on this point the simplest form arising out of the breakdown mechanisms has been used. Nor necessarily will there prove to be valid generalisations about the forms actually existing; oxygen substitution in particular, changes the number of classical structures available for each possible form, and so changes the energy relationships of the forms.

If pathways A and B do represent correct modes of breakdown for these compounds, various verifiable conclusions follow.

(i) As breakdown depends on form I(b), if forms I(a) or I(c) could be stabilised then fragmentation would be inhibited. Methylation stabilises I(a) and the behaviour of 4-methoxy-3-phenylcoumarins on electron bombardment is presented in a separate section of this paper. In fact, neither paths A or B are observed, as predicted. Only a pathway analogous to path C can be seen.

(ii) The hydrogen atom originally attached to the C_4-hydroxyl group of the substituted coumarin is bonded to C_3 in the tautomer I(b). Fragmentation by path A leaves this hydrogen atom on the phenylketene (II) whilst if path B is followed, it attaches itself in fragment (IV) to what was ring A of the flavonoid nucleus. These mechanisms imply a total specificity in this respect and so a ready test is available. Due to the high acidity of the 4-hydroxyl group deuterium exchange experiments are easily carried out and the products may be introduced into a previously deuterated mass spectrometer to see whether the appropriate fragments are raised by one mass unit. In each case this has been found to be so and in addition cleavage by path B leads to no appreciable isotope effect. Paths A and B are thus proven. This is of some importance as later papers will emphasise the generality of analogies to paths A and B in the mass spectra of many types of oxygen heterocyclic compound.

Section I.

Figure 1. 5,7-Dimethoxy-4-hydroxy-3-phenylcoumarin.

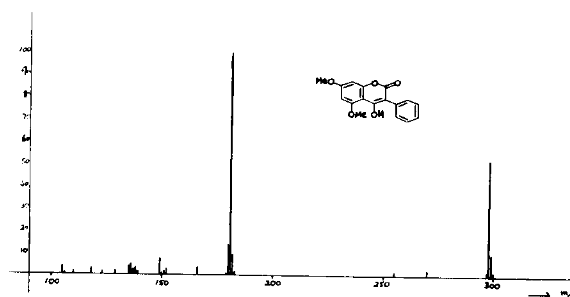


TABLE I

m/e	Intensity	m/e	I.	m/e	I.	m/e	I.
300	1.5	182	9.5	150	1.0	129	2.0
299	10.0	181	100	149	7.5	128	0.8
298	52.0	180	14	139	1.5	127	0.8
297	1.5	179	1.0	138	3.5	123	1.5
270	2.5	166	3.5	137	2.5	118	3.0
255	1.7	152	2.8	136	5.0	110	1.5
183	1.5	151	1.5	135	4.0	106	1.0
						105	4.0

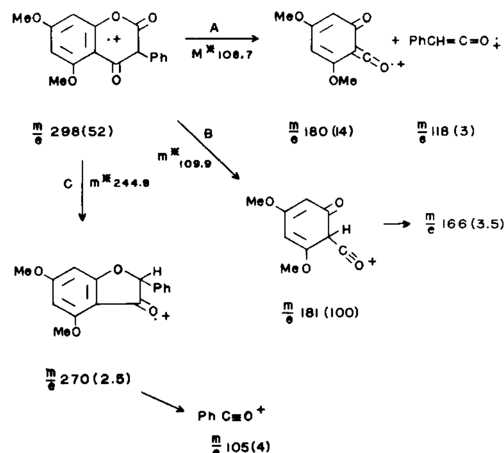


Figure 2. 4,4'-Dihydroxy-5,7-dimethoxy-3-phenylcoumarin.

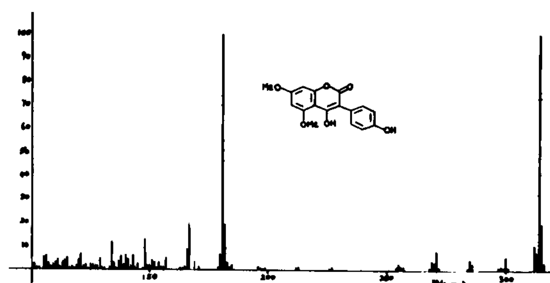
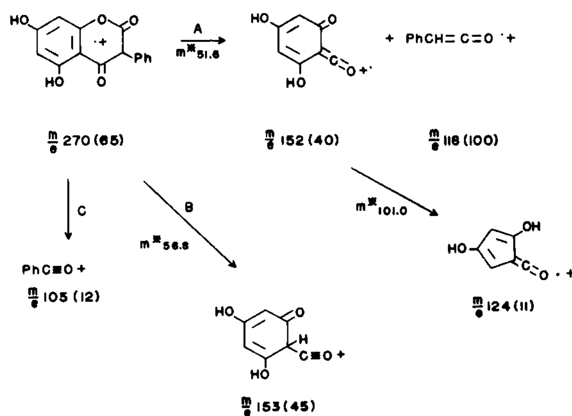


TABLE II

m/e	I	m/e	I	m/e	I	m/e	I	m/e	I	m/e	I
316	3.5	269	4.2	166	9.0	141	4.9	126	2.4	111	4.8
315	20	255	3.0	157	5.4	140	6.5	125	2.7	110	3.2
314	100.0	185	2.5	154	3.3	139	2.5	123	2.7	109	2.6
313	8.0	183	3.5	152	3.9	138	6.0	122	2.2	107	3.1
312	11.0	182	19.5	151	4.2	137	4.0	121	7.0	106	6.0
300	6.0	181	100.0	150	2.3	135	3.3	120	4.6	105	5.6
286	3.0	180	7.0	149	2.3	134	12.0	119	2.1	101	2.7
285	4.8	169	4.2	148	13.0	129	5.2	115	5.2		
271	8.0			145	3.0	128	2.1	114	4.4		
270	3.8	167	20.0	143	6.5	127	2.2	113	3.3		



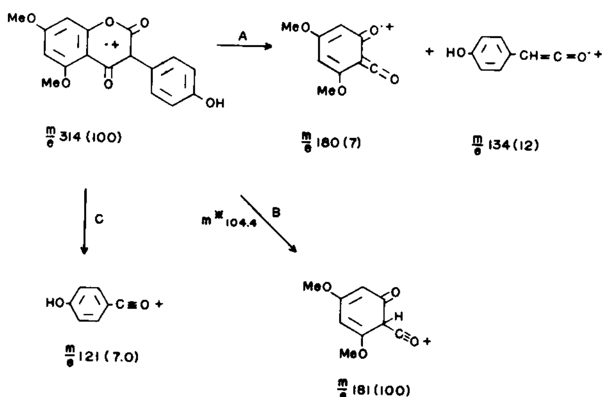


Figure 4. 4, 5, 7-Trihydroxy-4'-methoxy-3-phenylcoumarin.

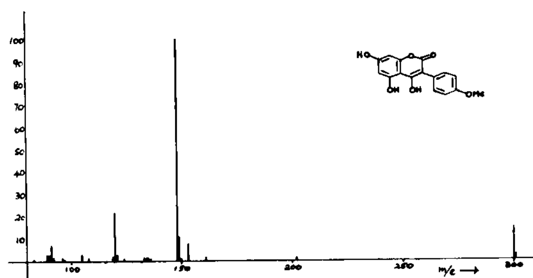


Figure 3. 4, 5, 7-Trihydroxy-3-phenylcoumarin.

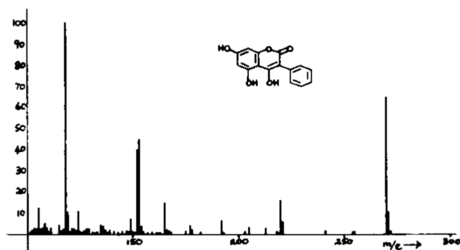
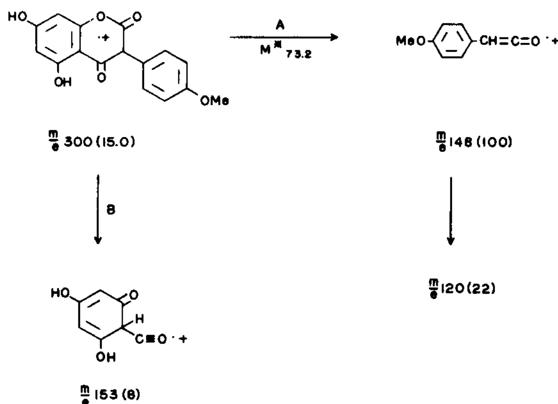


TABLE III

m/e	I	m/e	I	m/e	I	m/e	I	m/e	I	m/e	I
272	1.7	192	6.7	157	1.1	139	2.0	124	11.0	108	5.2
271	11.0	182	1.2	155	1.4	138	1.0	123	2.1	107	3.0
270	65.0	178	2.5	154	4.0	137	2.0	122	3.0	106	2.5
255	1.5	177	3.8	153	45.0	136	4.0	121	3.0	105	12.5
254	1.3	175	1.4	152	40.0	135	4.5	120	1.3	104	2.7
242	2.2	168	1.2	151	1.0	133	1.4	119	10.5	103	3.0
221	6.0	167	1.8	150	1.3	132	1.0	118	100.0	102	2.0
220	16.2	166	2.3	149	7.5	131	1.2	117	2.4	101	1.2
219	1.2	165	15.0	148	1.7	129	3.0	116	1.5		
218	1.7	163	1.1	147	2.0	128	2.8	115	4.5		
213	3.2	162	1.1	145	1.3	127	2.0	111	3.1		
203	1.5	161	1.1	143	1.2	126	1.1	110	1.4		
193	1.0	159	1.0	141	1.4	125	1.2	109	3.2		

TABLE IV

m/e	I	m/e	I	m/e	I	m/e	I
301	2.6	136	1.2	108	1.5	89	3.0
300	15.0	135	1.6				
202	1.5	134	1.4	105	3.2		
161	1.4	133	1.4	97	1.0		
153	7.8	124	1.2	96	1.8		
150	1.8	121	2.8	92	1.8		
149	10.7	120	22.0	91	7.4		
148	100.0	119	2.0	90	3.0		



Section II.

Figure 5. 4, 5, 7-Trimethoxy-3-phenylcoumarin.

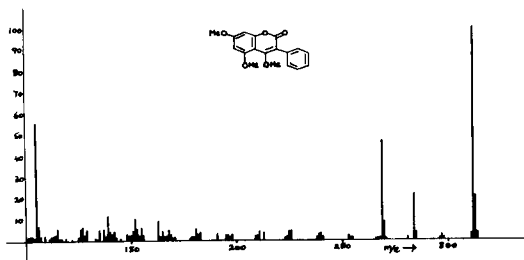


TABLE V

m/e	I	m/e	I	m/e	I	m/e	I	m/e	I
314	4.0	241	2.0	181	5.8	150	2.7	122	1.0
313	21.0	240	3.6	180	2.1	149	2.4	119	1.4
312	100	239	3.2	179	2.1	148	3.2	117	1.2
311	3.3	238	1.4	178	1.1	145	1.3	116	1.2
298	1.5	226	4.8	171	1.5	143	1.5	115	6.0
297	2.8	225	4.7	170	1.4	142	4.8	114	3.0
296	1.2	224	2.2	169	3.0	141	3.1	113	2.8
285	4.0	223	1.5	168	5.2	140	4.5	112	2.2
284	22.0	213	3.8	167	2.5	139	12.0	111	1.3
281	1.6	211	4.4	166	2.2	138	2.6	109	2.4
271	1.0	210	2.4	165	4.7	137	5.6	107	2.2
270	8.8	209	2.3	164	2.0	135	5.2	106	7.0
269	47.0	198	3.0	163	9.5	134	1.0	105	56.0
268	1.4	197	2.0	156	2.8	133	1.3	104	1.7
267	1.2	196	2.8	155	6.2	129	5.5	103	2.5
266	1.1	195	2.8	154	2.4	128	2.8	102	2.6
255	1.3	191	3.2	153	5.7	127	6.9	101	2.0
254	1.3	183	4.2	152	10.5	126	5.6		
253	2.6	182	3.2	151	4.8	125	1.8		

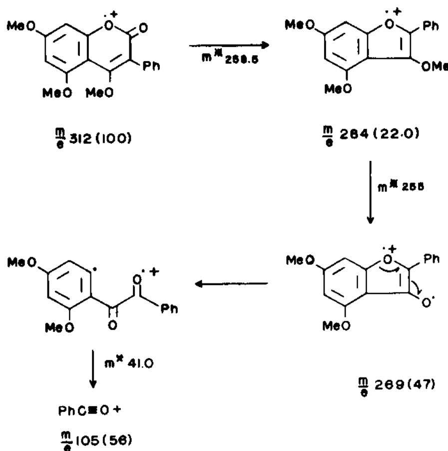


Figure 6. 4, 4', 5, 7-Tetramethoxy-3-phenylcoumarin.

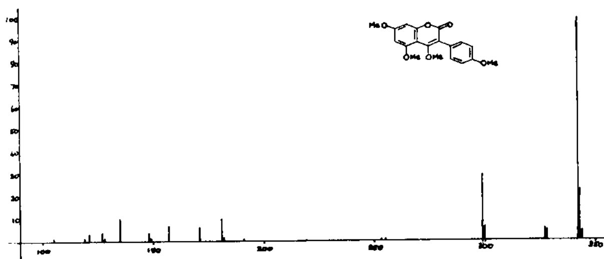
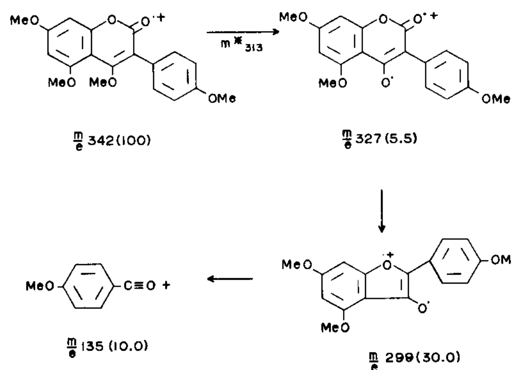


TABLE VI

m/e	I	m/e	I	m/e	I	m/e	I	m/e	I	m/e	I
344	4.5	327	5.5	255	1.1	182	1.6	148	4.0	121	3.5
343	23.0	314	1.0	253	1.0	171	6.5	135	10.0	119	1.5
342	100.0	300	6.5	191	1.0	157	7.0	128	4.0	105	1.4
328	5.0	299	30.0	181	10.0	149	1.6	127	1.6		



Section III.

Figure 7. 7-Hydroxy-4-methylcoumarin.

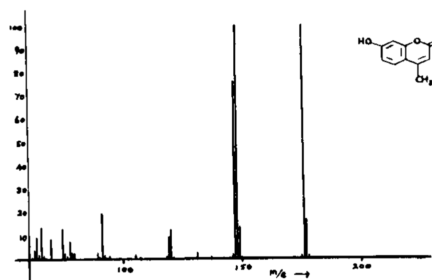


TABLE VII

m/e	I	m/e	I	m/e	I	m/e	I	m/e	I	m/e	I
178	2.0	148	100.0	121	1.4	105	2.5	92	2.3	78	3.4
177	17.0	147	76.0	120	13.0	103	1.1	91	20.0	77	8.0
176	100.0	146	2.5	119	10.0	102	1.2	90	1.6	76	2.2
175	2.0	137	1.5	118	2.0	94	1.9	89	3.5	75	3.0
150	1.5	131	3.2	107	1.3	93	1.0	79	3.4	74	13.5
149	14.5										

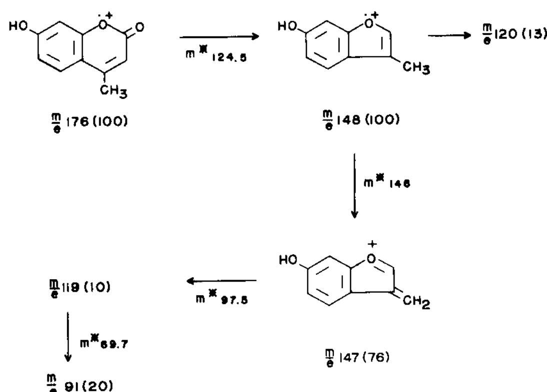


Figure 8. 4,7-Dihydroxycoumarin.

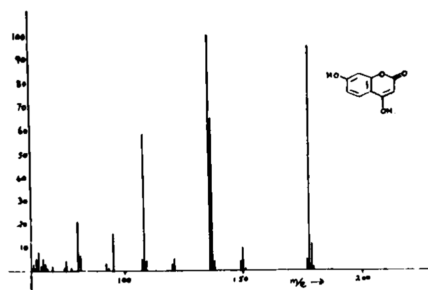
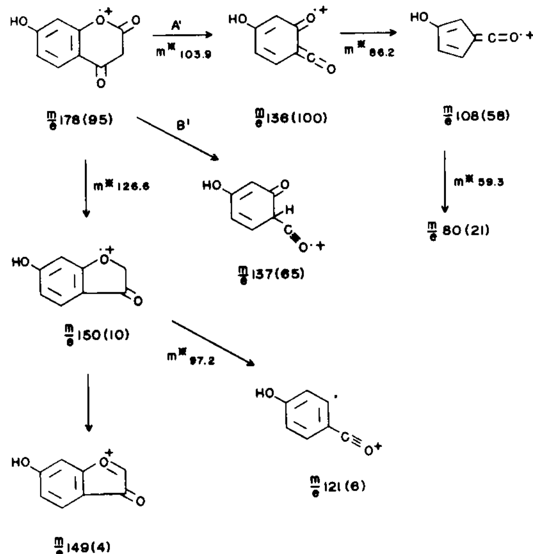


TABLE VIII

m/e	I	m/e	I	m/e	I	m/e	I	m/e	I	m/e	I
180	2.0	149	4.5	120	2.9	93	1.6	75	4.5	65	5.3
179	12.0	138	5.4	109	4.6	92	3.5	74	1.7	64	2.6
178	95.0	137	65.0	108	58.5	81	7.0	69	2.3	63	8.2
177	5.0	136	100.0	107	5.1	80	21.0	67	1.2	62	5.6
150	10.0	121	5.3	95	16.0	77	1.8	66	3.1	61	3.3



Section IV.

An example of a deuteration experiment was the deuteration of dihydrorobustic acid, whose spectrum is presented below. For the purpose in hand it is sufficient to concentrate attention on the peaks at m/e 235 and m/e 234 arising as shown.

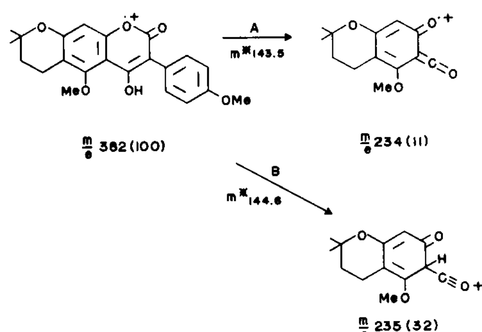
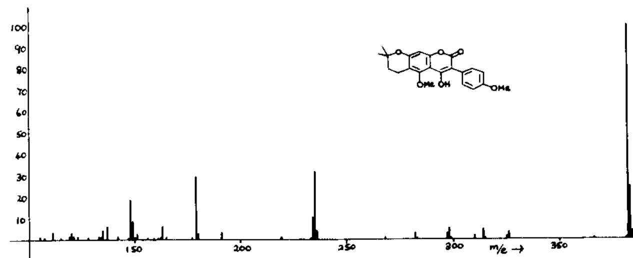
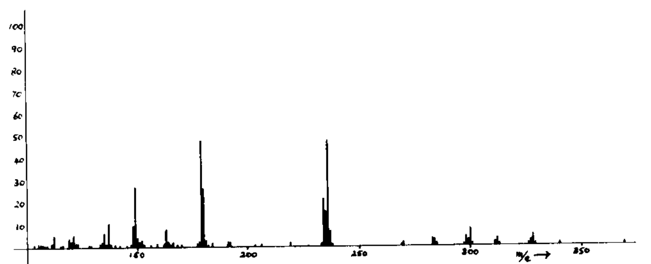


Figure 9.



Deuteration followed by fragmentation by path A should lead to m/e 234 from both deuterated and undeuterated species, but when path B is followed the deuterated dihydrorobustic acid should give rise to a peak at m/e 236. The spectrum of partially deuterated dihydrorobustic acid was as shown the peak at m/e 382 corresponds to

Figure 10.



undeuterated dihydrorobustic acid and has an intensity of 26.

The peak at m/e 383 corresponds to deuterated dihydrorobustic acid plus a contribution due to the isotope peak from m/e 382, amounting to 6.33 units, and has an intensity of 100. $\therefore$ % of deuterated dihydrorobustic acid in mixture is $\frac{93.67 \times 100}{93.67 + 26} = 78.3\%$

% of dihydrorobustic acid is $\frac{26 \times 100}{93.67 + 26} = 21.7\%$

The peak at m/e 234 of intensity 21.5, arising from both species of dihydrorobustic acid makes an isotopic contribution to m/e 235 of $\frac{21.5 \times 14.43}{100} = 3.10$ and to m/e 236 is $\frac{21.5 \times 1.77}{100} = 0.4$.

The peak at m/e 235 has an intensity of 16, which becomes 12.9 when corrected for the isotopic contribution from m/e 234. The isotope contribution of this corrected m/e 235 peak to the m/e 236 peak (intensity 48.0) is $\frac{12.9 \times 14.40}{100} = 1.9$. Therefore, the true intensity of the m/e 236 peak is 48.0 - 1.9 - 0.4 = 45.7.

The monoisotopic ratios are summarised:

m/e 234 ($C_{13}H_{14}O_4$) = 21.5
m/e 235 ($C_{13}H_{15}O_4$) = 12.9
m/e 236 ($C_{13}H_{14}DO_4$) = 45.7

The peak at m/e 234 consists of contributions from both species, that from deuterated dihydrorobustic acid 0.783 x 21.5 = 16.83, and that from dihydrorobustic acid being 4.67.

$\therefore$ For deuterated dihydrorobustic acid $\frac{m/e\ 234}{m/e\ 236} = \frac{16.83}{45.7} = 0.368$

For the undeuterated species, $\frac{m/e\ 234}{m/e\ 235} = \frac{4.67}{12.9} = 0.362$

This latter ratio was also calculated from the spectrum of pure dihydrorobustic acid run under identical conditions and was found to have the value of 0.368, in excellent agreement with that found from the spectrum of the mixture.

The calculations demonstrate that in path B the deuterium is quantitatively and specifically transferred by the path shown, and that no isotope effect is observed.

Sample Introduction.

All spectra were taken on an AEI Ltd., MS 9 mass spectrometer, using a direct insertion probe. All the samples were introduced directly into the mass spectrometer (MS 9) ion source and evaporated into the ionisation region from the end of the sample probe situated only a few millimeters away. (The advantages of direct evaporation are now well known, mainly that the required sample temperatures are lower by 100-150° than those required by the reservoir system, thus reducing the amount of thermal decomposition). Rapid change-over of samples and convenience of adjusting evaporation rates were achieved with the vacuum lock system used for inserting the sample probe.

Particular features of the inlet system are:

(i) Samples can be changed in less than half a minute without any adverse effect on the vacuum.

(ii) The axis of the system is in a direct line with the electron beam, and the sample probe can be passed through a channel in the ion source block to a point as close as desired to the electron beam itself.

(iii) The sample probe, a loose item inside the main tube can be moved by a magnet outside the vacuum system. With this device the sample can be quickly introduced or removed from the ion chamber as desired so that evaporation can be suspended whilst reference compounds are introduced or instrumental performance adjusted or the next steps in the analysis considered.

(iv) The appropriate sample temperature is developed by thermal contact with the heated ion chamber block. This ensures that the temperature of the ion chamber is slightly higher than that of the sample, thus avoiding condensation. A considerable degree of adjustment of the sample temperature is achieved by small changes in the probe position using the magnet control.

REFERENCES

(1) A. Pelter and P. Stainton, *Tetrahedron Letters*, 1209 (1964).
(2) A. P. Johnson, A. Pelter, and M. Barber, *ibid.*, 1267 (1964).
(3) A. Pelter and A. P. Johnson, *ibid.*, 2817 (1964).
(4) C. S. Barnes and J. L. Occolowitz, *Australian J. Chem.*, **17**, 975 (1964).
(5) R. I. Reed and J. M. Wilson, *J. Chem. Soc.*, 5949 (1963).
(6) Unpublished observations by authors.

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