692. *Electron Impact and Molecular Dissociation. Part I. Some Steroids and Triterpenoids.*

By R. I. Reed.

The mass spectra of fragments of some steroids and triterpenoids produced by bombardment with electrons of 9—15 ev energy indicate thermal fission during introduction of the molecules into the ion-chamber. The scope and limitations of this technique are discussed, particularly for the determination of the length of the side-chain.

Experiments with 30—70 ev electrons permit study of dissociations induced by electron-impact and the spectra of cholestane, ergostane, lanost-9(11)-ene, and stigmastadiene fragments have been qualitatively interpreted.

Correlation of the ions obtained by the electron-induced dissociation with specific structural features in the molecules is of interest and importance. Previous studies, that of Beynon[1] being of particular note, have usually been directed[2] to identification of molecules by recognition of their distinctive fragmentations (cracking patterns).

The present investigation attempts to extend these methods to low-energy bombardment and to account theoretically for the principal fragments from some steroid and triterpenoid hydrocarbons.

Results

Low electron energies were generally used but in a few cases the much higher energies[3] commonly employed for the production of "cracking patterns."

The low-energy studies were carried out in order to determine molecular weights[4] and, as far as possible, the number of carbon atoms in the side-chain. The results are given in Table 1.

Table 1.

Compound	Mol. wt. (nominal mass units) Found	Calc.	Side-chain Found	Calc.
Cholestane	372	372	110	113
Ergostane	386	386	123	127
Stigmastadiene	396	396	—	—
Stigmastanone	413	414	137	141
α-Amyrin	411	410	None	None
Lanost-9(11)-ene	413	412	—	—

Table 2.

Cholestane		Ergostane		Lanost-9(11)-ene		Stigmastadiene	
Mass	Intensity	Mass	Intensity	Mass	Intensity	Mass	Intensity
372	Parent	386	Parent	412	Parent	396	Parent
357	M	371	M	397	VS	381	S
343·6	Metastable	357·4	Metastable	288	M	353	S
262	M	276	M	287	S	288	W
232	M	232	M	274	S	257	VS
217	VS	217	VS	273	M	149	M
149	S	149	S	149	M	139	M
110	W	110	W	139	M	127	M
109	S	109	S	110	W	108	W
95	S	95	S	109	S	43	VS
55	VS	55	VS	43	S		
43	VS	43	VS				

The high-energy studies were confined to lanost-9(11)-ene, stigmastadiene, cholestane, and ergostane. Table 2 lists only the most prominent peaks, roughly classified; at the

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[1] Beynon, *Nature*, 1954, **174**, 735; *Mikrochem. Acta*, 1956, 437.

[2] Gilpin and McLafferty, *Analyt. Chem.*, 1957, **29**, 990.

[3] Barnard, "Modern Mass Spectrometry," Inst. Physics, London, 1953, p. 193.

[4] de Mayo and Reed, *Chem. and Ind.*, 1956, 1481.

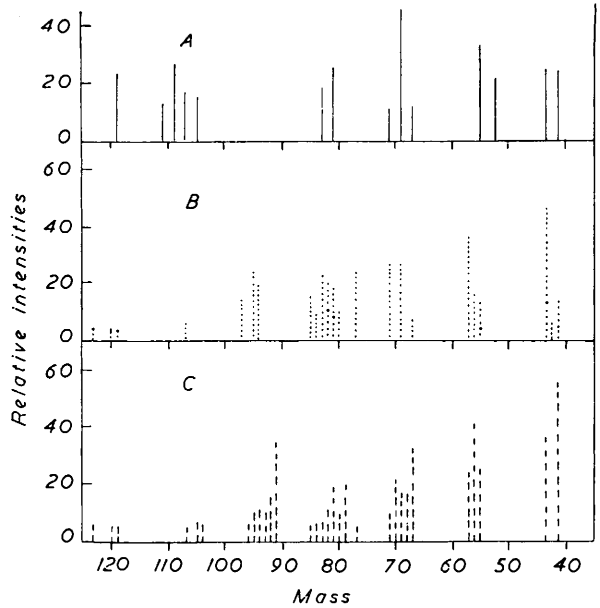
present stage, only a qualitative interpretation is justified. Agreement with published data is good. I am indebted to Mr. A. Quayle [5] for identification of the metastable peaks here reported, also for an assessment of many of the peak strengths which in general corroborate the present results.

DISCUSSION

The observations suggest two effects: one, which predominates at low accelerating voltages, appears to be mainly a thermal effect; the other, at high electron energies, is an electron-induced dissociation.

Low-energy Spectra.—The characteristic of the thermal mechanism is that the side-chain and fragments of it are probably produced either by the heating of the sample or by a combination of this with the low-energy electron beam. This is important, for a fragment corresponding to the mass of the side-chain, or of this mass less some hydrogen atoms, should be obtained. This will be the greatest mass in a series of particles of relatively low molecular weights corresponding to fragments of the hydrocarbon chain. In favourable cases such as those here enumerated, where the ring-skeleton is not ruptured by the conditions of experiment, confusion between the ions corresponding to fragments of the chain (terminating in a mass corresponding to the complete side-chain) and others derived from the nucleus or the nucleus plus remnants of the side-chain is unlikely. The lower series will not in general correspond to an alkyl chain of more than ten carbon atoms whereas, in the steriod and triterpenoid fields, the nucleus contains at least seventeen carbon atoms.

FIG. 1. *A, High-energy spectrum. B, Low-energy spectrum obtained by conventional methods. C, Low-energy spectrum obtained from a specimen first heated with a platinum wire.*



Under the low-energy conditions there is insufficient energy in the electron beam both to fragment and to ionise the molecule, at least to any great extent, and thus the breakdown pattern from the parent hydrocarbon is rather simple. Electron-induced dissociations are of a rather low intensity, those yielding fragments formed by multiple bond-fissions being particularly weak, whilst the ions derived from the ionisation of the thermally produced particles are more abundant. Thus for cholestane the spectrum in our

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[5] A. Quayle, personal communication.

experiments at low energies consists of a sparse series of peaks up to a mass of ~109 corresponding to an eight carbon fragment and then peaks of only very low intensity for masses less than 230, which correspond to the ring skeleton.

Fig. 1 shows the difference between low- and high-energy spectra for lanost-9(11)-ene in which the mass of 113 units corresponds to the complete side-chain.

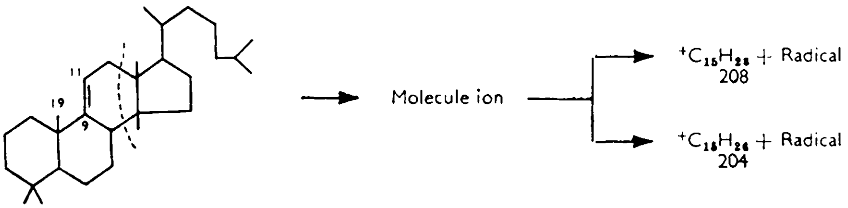
The technique described, therefore, is in favourable circumstances well suited to the determination of molecular weights on very small amounts of material, enabling a ready distinction to be made between steroids and triterpenoids. Moreover, the tetracyclic triterpenoids can be easily distinguished, by means of the side-chain estimation, from the pentacyclic members.

For substances such as α-amyrin which lack a side-chain the above pattern is much altered. There is no series corresponding to pyrolysis of the side-chain and there is virtually no spectrum until the electron-beam energy is ~18 ev, when a spectrum characteristic of the electron-induced dissociation appears. This spectrum has ions corresponding to nearly all mass-numbers for an integral number of carbon atoms with the appropriate number of hydrogen atoms. This spectrum is not further considered in this communication.

Evidence has been adduced by Brown and Gillams [6] which supports the view that molecule ions containing double-bonds tend to break preferentially at linkages β to the unsaturated centre. Studies upon the isomeric octanes [7] and nonanes [8] have also shown ready loss of a carbon chain from a quaternary centre. Additionally, the reasonable assumption is made that ions formed by breaking of one single bond will be more abundant than those which require fission of two or more such bonds.

These generalisations enable some predictions to be made concerning the main features of the " cracking-patterns " in the following molecules.

In lanost-9(11)-ene, the ready elimination of a methyl group, either the allylic $C_{(19)}$ or one of the *gem.*-dimethyl groups, would be expected. The bond-fission illustrated should also occur, yielding fragments of mass 204 and 208. The possibility of a further break-down of the radicals as well as rearrangement [9] is to be understood. The fragment of mass 204, containing the double bond, may be the more intense but, as either may gain a hydrogen, a process frequently associated with the breaking of bonds in the molecule ion,[10] or perhaps eliminate a further hydrogen atom, all that may be predicted is that an intense peak should occur in the spectrum within one or two mass units of 204—208. Alternatively, by the breaking of the allylic bonds in rings A and B masses of 287 and 125 may be obtained.



The experimental evidence agrees with this, the ion of mass 397 being the strongest in the spectrum. Moreover, ions of mass 288 and 274 are present which although relatively weak in intensity are unusually strong for fragment ions of even mass. The former may be readily interpreted as a hydrogen addition to mass 287, or by the loss of one methyl group and the entire side-chain from the original molecular ion. Mass 274

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[6] Brown and Gillams, A.S.T.M. Committee E-14 on Mass Spectrometry, 2nd Annual Meeting, New Orleans, 1954.

[7] Bloom, Mohler, Lengel, and Wise, *J. Res. Nat. Bur. Stand.*, 1948, **41**, 129.

[8] Mohler, Williamson, Wise, and Wells, *ibid.*, 1950, **44**, 291.

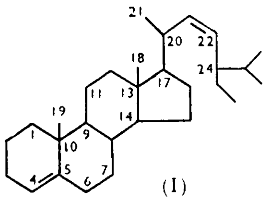
[9] Stevenson, *Trans. Faraday Soc.*, 1953, **49**, 867.

[10] McLafferty, *Analyt. Chem.*, 1956, **28**, 306.

can be obtained from this by the further loss of a methylene group. The fairly abundant ion 149 may be obtained by further break-down of one of the larger ions.

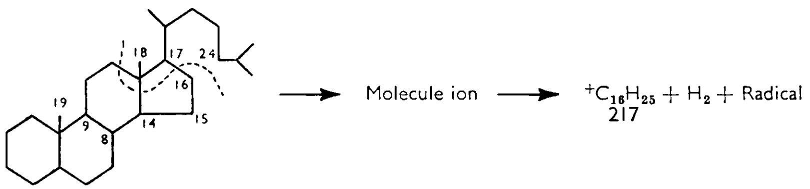
It is the occurrence of secondary reactions such as hydrogen transfer and the possibility that there is more than one origin of the fragment in the molecule which make the interpretation of fragments even of high mass difficult, and of low mass still more difficult.

The very abundant ions at masses 381, 353, 257, and 139 may be predicted for stigmastadiene (I) corresponding to the fission of the bonds at $C_{(21)}$ or $C_{(19)}$, $C_{(24)}$, and $C_{(18)}$ respectively, which is in agreement with observation except that the ion 139 corresponding to the entire side-chain is weaker than the others. Bond-fissions at positions 6 : 7 and 9 : 10 would yield rather weaker ions of masses 108 and 288 of which the latter is reduced to 149 by further loss of the side-chain. Alternatively the two-fold fission at positions 1 : 10 and 2 : 3 would give a mass of 368 which on losing the side-chain becomes 127. Peaks of moderate intensity have been observed for all of these.



(I)

The case of the homologous, saturated hydrocarbons cholestane and ergostane is more complex, as these do not possess the necessary allylic system. The most intense amongst the peaks for higher mass is 217 for both hydrocarbons, which excludes the side-chain beyond $C_{(24)}$ from the ion if it has the same source in each molecule. This has previously been interpreted as a fission which removes the side-chain and parts of rings C and D, as shown, for cholestane:



A second intense fragment common to these compounds has a mass fifteen units greater than this, which will require a quite different explanation. An alternative theory is here proposed in which the 8 : 14 bond in the molecule ion of these steranes behaves as an ethylenic double bond. No direct evidence is available as to this bond length under these conditions, but there is some chemical evidence that this is one of the more stable positions in the molecule for an ethylenic linkage, in that under certain conditions of catalytic hydrogenation, migration to this position of double bonds from positions 7 : 8 and 8 : 9 is known to occur,[11] which suggests that a more stable molecular structure is obtained by the shortening of the 8 : 14-distance.

With this assumption, the predicted fragment ions of cholestane would be 357, 262, and 110, 232, 217, 149, and 109 corresponding respectively to the elimination of the 18-methyl group and the multiple bond-fissions 6 : 7, 9 : 10, 13 : 17, and 15 : 16. The further loss of one hydrogen gives mass 109 and the elimination of a methyl group from 232 yields 217. The ion of mass 149 may be derived from the loss of the side-chain from the fragment 262. The observed results are in accordance with this. Similarly the corresponding ions, predicted and observed, of mass 371, 276, and 110, 232, 217, 149, and 109 may be obtained from ergostane. It is accordingly suggested that the above postulate provides the most economic explanation of the observed fragmentations.

The ion of mass 43 is fairly prominent in all the spectra discussed and can, moreover, be readily interpreted as due to removal of the terminal *iso*propyl group from the side-chain. However, in view of the many possibilities for producing a three-carbon fragment from such molecules, evidence of this nature is permissive rather than compelling.

[11] Rodd, "The Chemistry of Carbon Compounds," Elsevier Publ. Co., London, 1953, Vol. IIB, p. 931.

EXPERIMENTAL

The samples were generally analysed by volatilising the solid into the ion-chamber of a Metropolitan-Vickers M.S.2. mass-spectrometer. The electron-energy was varied from ~30 to 70 ev by using a stabilised emission current and for electron energies from 9 to 20 ev without current stabilisation. For masses up to 200 an accelerating potential of 2000 v was used which was lowered to 1000 and exceptionally 800 v for higher masses up to ~430. At this low accelerating voltage, even with the smallest practicable slit width at the collector, the resolution was poor. In all cases the spectrum was scanned by varying the field strength of the magnet.

The material was volatilised in the furnace shown in the diagram. This furnace consisted of a small copper tube some 3 mm. deep and having a 3 mm. cross-section. The bottom of the tube was closed by a sintered disc whilst the top was threaded to receive a threaded copper rod. The material under investigation was placed in the container which was then screwed on to the copper rod.

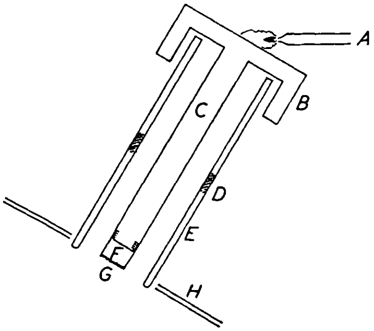


FIG. 2.

A, Micro-burner. B, Copper block on the end of the copper rod forming a gas-tight seal. C, Copper rod. D, Glass–metal seal. E, Glass inlet tube. F, Sample chamber. G, Terminal sinter. H, 2000 v, *Repeller plate of the ion chamber.*

The copper rod which had a diameter of about 2 mm. was some 9 cm. long and at the further end terminated in a small copper block. This assembly was inserted into the gas-inlet tube of the ionisation head and the copper block screwed on to the upper end of this inlet to form a gas-tight joint. The length of the total assembly was such that the sintered disc was level with the exit end of the inlet-tube in the proximity of the electron beam.

In general the samples, particularly those melting below 60°, were sufficiently volatile to provide an adequate vapour pressure for the production of a "cracking pattern." Exceptionally, heating was required which was achieved by heating the copper block by means of a gas micro-burner, the heat being conducted by the copper to the substance. The apparatus was tested on anthracene and phenanthrene, both giving identifiable mass-spectra when heated in the above furnace.

For the low-energy studies it was necessary to obtain a high-concentration of vapour because of the low efficiency of ionisation under these conditions. The samples were heated at 350—400° for 5—10 min. in a small container by gas or by a platinum wire in contact with the substance.

These compounds were then admitted to the mass-spectrometer through a sinter, which acts as a molecular leak, in the conventional manner. In order to obtain the most intense spectra possible, the machine was run at the highest sensitivity and accordingly only the relative intensities of the fragments have any significance.

I thank Professor D. H. R. Barton for advice, encouragement, and provision of samples, also Dr. P. de Mayo for the benefit of much discussion. I am also very grateful to " Shell " Research Ltd. and Mr. A. Quayle, of their Thornton Research Centre, for the provision of spectra obtained under much higher resolution than was available to me.

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