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Mass Spectrometry of Cannabinoids

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Abstract □ The mechanism of fragmentation of cannabinoids to fragments *m/e* 314, 299, 271, 258, 246, 243, and 231 is given. Cannabidiol, cannabinodiol, cannabinol, Δ^6- and Δ^1-tetrahydrocannabinol, cannabichromene, cannabicyclol, derivatives with pentyl, propyl, and methyl side chains, their methyl ethers, and *cis–trans* and *ortho–para* isomers were analyzed by GLC–mass spectrometry using different energies for fragmentation during GLC elution. The following mechanism was distinguished: loss of a methyl radical, ring closure and rotation, McLafferty rearrangement, retro Diels–Alder, internal protonation, isomerization and internal bond formation, and one-step fragmentation to *m/e* 231.

Keyphrases □ Cannabinoids, various—mechanism of mass spectrometric fragmentation □ Mass spectrometry—mechanism of fragmentation of various cannabinoids

One rapid method of identification of cannabinoids is combined GLC–mass spectrometry. The mass spectra of cannabinoids can be distinguished from each other. The fragmentation process is relatively slow for most compounds, taking 40–50 ev before completion, after which the relative intensities of the fragments do not alter (Fig. 1). When the relative intensities of each particular mass fragment are plotted against the electron energy used, the fragmentation process can be characterized for each cannabinoid.

In the 10–20-ev range, the fragmentation just starts; differences in this process between the different structures are then relatively large. The technique of taking mass spectra at different electron voltages between 10 and 20 ev and plotting relative intensities against electron volts gives very characteristic electron voltage mass fragmentograms of the cannabinoids, which, for instance, led to the discovery of some propyl and methyl homologs of the known cannabinoids. This method is also useful in elucidating the various cannabinoid fragmentation pathways.

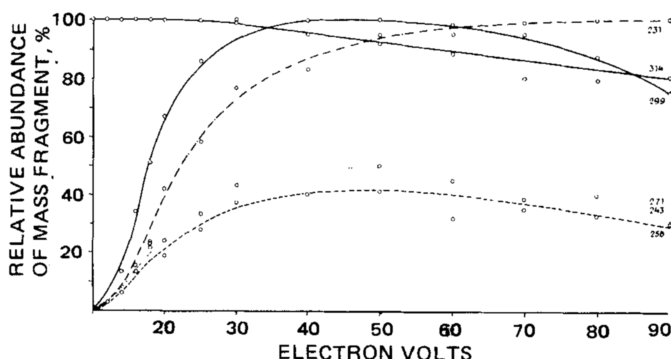


Figure 1—*Electron voltage mass fragmentogram of* trans-para-Vb. *Fragments are formed relatively slowly between 10 and 30 ev. In this particular interval, small structural differences of the cannabinoids may result in big differences in the formation rate of certain mass fragments.*

EXPERIMENTAL

Materials and Methods—A gas chromatograph–mass spectrometer[1] was used. Glass columns, 1.80 m × 4 mm i.d., were packed with 3% OV-17 on 60–80-mesh Gas Chrom Q. The temperatures were: oven, 200°; separator, 220°; and ion source, 250°. The helium flow was 20 ml/min. Repetitive mass spectra were taken at 20, 18, 16, 14, 12, and 10 ev during the elution of a GLC peak. The detector gain was increased in the 16–10-ev energy interval. The trap current was 60 μamp, and the accelerating voltage was 3.5 kv. The pressure in the analyzer tube was 2×10^{-6} Torr.

The gas chromatograms were recorded by a total-ion current monitor at 20 ev. Gas chromatograms of mixtures of natural and synthetic cannabinoids were published elsewhere (1). The mass spectra obtained were normalized, and the relative abundance of a particular mass fragment was plotted against the electron voltage.

Cannabinoids[2]—Lebanese, Nepalese, Moroccan, Columbian, Indonesian, and Congolese hashish samples and marijuana samples from Brazil and South Africa were powdered and extracted with ether or *n*-hexane by homogenizing for 10 min. After filtration of the extracts, most of the solvent was evaporated to give suitable concentrations for GLC–mass spectrometry. Synthetic samples were only dissolved in a small aliquot of ether.

RESULTS

Molecular Ion *m/e* 314—The aryl nucleus is the center of charge localization, and elimination of an electron from this nucleus gives the molecular ion *m/e* 314. At 10 ev, the molecular ion *m/e* 314 is the base peak. At 20 ev, large differences in the relative intensities between different cannabinoids can be observed (Table I).

Formation of Mass Fragment M − 15 (*m/e* 299 and 295)—The loss of the geminal methyl group, which nearly all structures have in common,

[1] LKB 9000.
[2] Natural Cannabis samples were obtained from and identified by Dr. A. H. Witte, Laboratory of Forensic Sciences, Ministry of Justice, The Hague, The Netherlands, and Dr. E. A. Carlini, Depto de Psicobiologia, Escola Paulista de Medicina, Sao Paulo, Brazil. Synthetic cannabinoids were a gift from Dr. T. Petrzilka, Eidgn. Hochschule Zurich, Department of Organic Chemistry, Zurich, Switzerland, where voucher specimens were deposited.

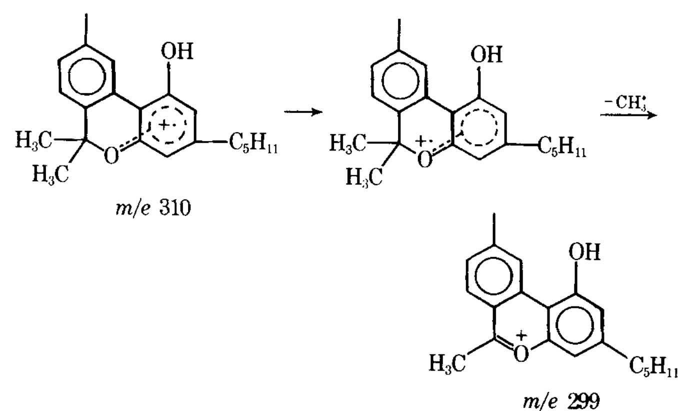
Table I—Relative Intensities of Molecular Ion *m/e* 314 of Different Cannabinoids Taken at 10 and 20 ev

Compound	10 ev	20 ev	Electron Volts of 50% Relative Abundance
Cannabicitran (I)	100	10	14
Cannabicyclol (II)	100	10	14
Cannabichromene (III)	100	10	11
Cannabidiol (IV)	100	25	16
Δ^6-*trans*-Tetrahydrocannabinol (V*a*)	100	100	—
Δ^1-*trans*-Tetrahydrocannabinol (V*b*)	100	100	—

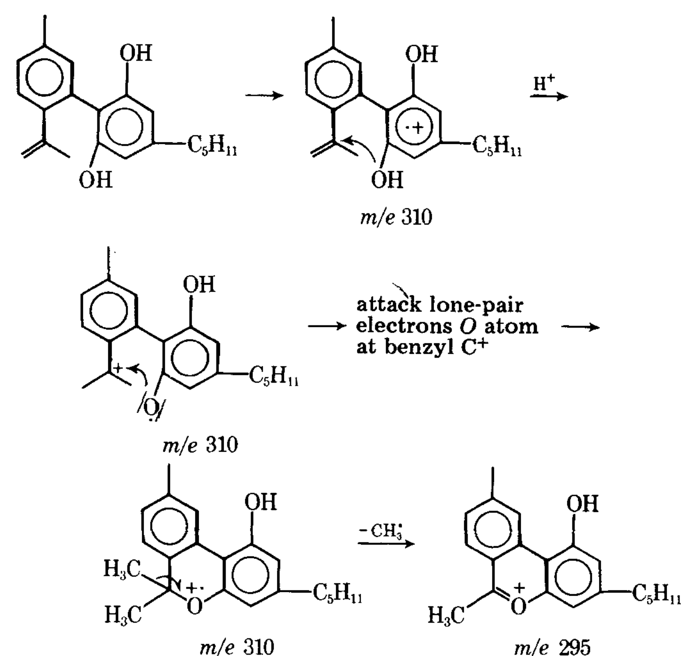
depends strongly upon the structure of the compound. In cannabinol (VI), the main process is this particular fragmentation; other possible fragmentations are ruled out or contribute very little (2). The relative intensity of M − 15 increases when the double bond moves toward the aryl nucleus (Table II). When the double bond is in the 3-4-position as in synhexyl (IX), the only fragmentation of the alicyclic part is loss of a geminal methyl group (3) (Fig. 2). Substitution in the aryl nucleus has no influence on this fragmentation (Figs. 2 and 3).

The presence of the pyran ring is necessary for this kind of fragmentation. For IV, *m/e* 299 originates after ring closure (4), but this mechanism has strong competition with the retro Diels–Alder reaction and a one-step mechanism (5). This process is is also the most logical for the aromatic VII (3) (Schemes I and II).

With a series of derivatives of tetrahydrocannabinol [Δ^6 (V*a*), Δ^1 (V*b*),



Scheme I



Scheme II

Table II—Relative Intensities of the M − 15 Fragment of Several Cannabinoids

Compound	Relative Abundance at 20 ev	Electron Volts of 50% Relative Abundance	Total Ion Current at 20 ev, %
II	5	—	3
III	5	—	3
IV	5	—	3
V*a*	10	—	5
V*b*	60	18	22
VI	100	12	83
Cannabinodiol (VII)	100	16	44

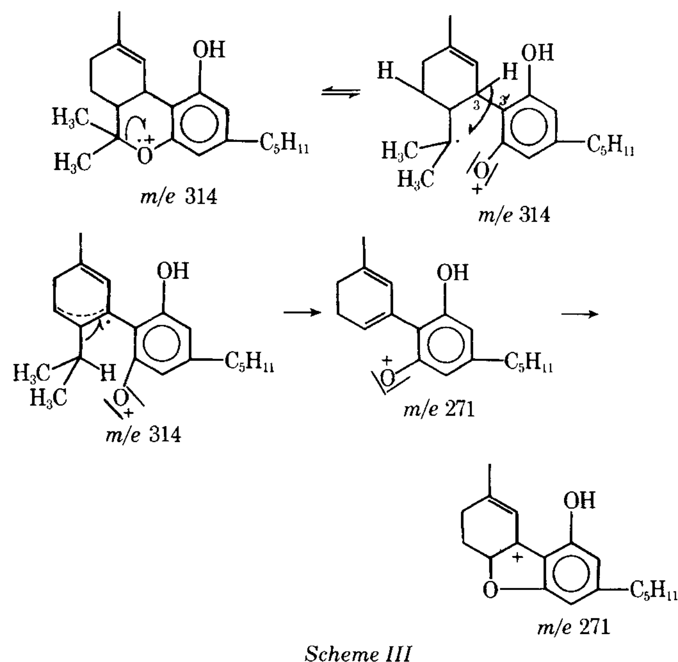
Δ^2 (V*c*), and Δ^3 (V*d*)], the loss of a geminal methyl group became the main process when all competing fragmentations were no longer possible (2). This influence of the double bond on the mass spectra might be used for the identification of tetrahydrocannabinol isomers because the ratios of relative intensities of M − 15 and M of the isomers were characteristic. The ratios were 0.13, 0.81, 2.8, 7.3, 5.5, 1.1, and 6.5 for V*a*, V*b*, V*c*, V*d*, VI, VII, and IX, respectively. The mechanism given in Scheme I is based on the concept of charge localization at the oxygen atom.

With 7,7,7-trideutero-V*a*, loss of a methyl radical resulted in equal relative abundances of *m/e* 299 and 302 (5). This observation leads to the conclusion that the methyl group at position 7 is lost and that the 1–6-double bond (Δ^6, V*a*) acts as a competitive center of charge localization. When the double bond moves toward the aryl nucleus, it becomes part of the main center of charge localization and the loss of the geminal methyl group becomes the main fragmentation process.

Formation of Mass Fragment M − 43 (*m/e* 271 and 285)—Mass fragment *m/e* 271 may be formed according to Scheme III (4). After ring opening, the possibility of hydrogen transfer or ring reclosure exists.

When the ring is open, rotation around 3-3′ axis may take place. If two hydroxyl groups are available for the reclosure, there is no difference in the position of the aromatic nucleus. If the alkyl side chain is in the *ortho*-position, the chance of ring reclosure statistically is only 0.5. The influence of the *ortho–para*-position of the side chain on the formation of *m/e* 271 is negligible, except in the case of Δ^6-tetrahydrocannabinol (Table III). Even methylation of the phenolic hydroxyl groups has no influence on the formation of *m/e* 271 (*m/e* 285). With 3-^{2}H-Δ^6-tetrahydrocannabinol, complete retention of the deuterium label was found (*m/e* 271 → *m/e* 272) (5). The finding that *m/e* 271 in Δ^1-*cis*-tetrahydrocannabinol is 100% for both *ortho*- and *para*-isomers may be explained by the relief of tension of the folded structure. Ring opening is, therefore, favored.

This energetically favored ring opening is not supported by the formation of *m/e* 271 in Δ^1-*cis*- or *trans*-tetrahydrocannabinol. If the concept of charge localization in the aromatic nucleus is maintained, then



Scheme III

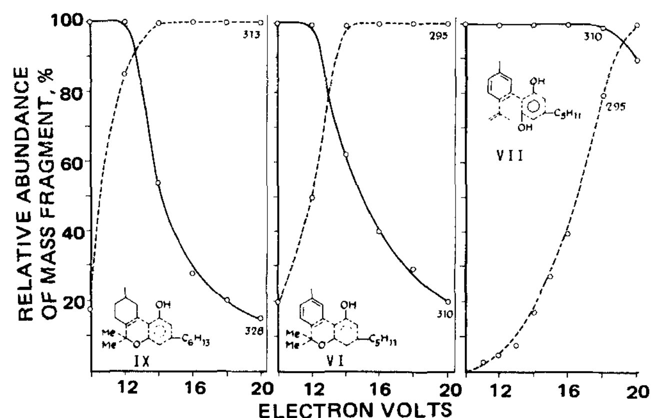


Figure 2—*Electron voltage mass fragmentograms of IX, VI, and VII.*

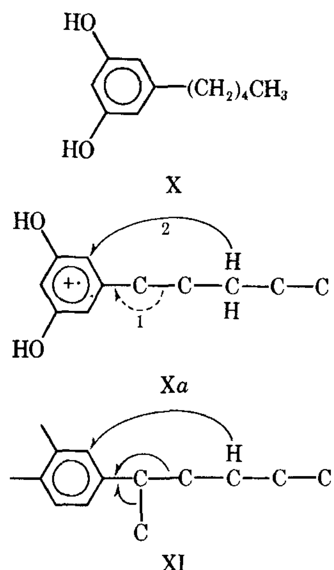
a shift of the hydrogen at C-5 can solve the problem of the formation of *m/e* 271. However, V*a* and V*b* isomers (*cis–trans–ortho–para*) deuterated at C-5 must be synthesized to give absolute proof of the mechanism.

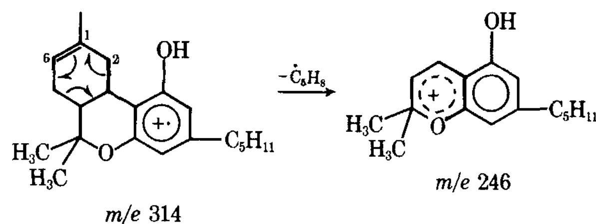
Formation of Fragment M − 56 and Fragmentation of Side Chain (*m/e* 258)—The side chain is not fragmented carbon for carbon successively. Olivetol (X) itself shows the following mass spectra at 20 ev: *m/e* (relative abundance) 180 (M, 37), 165 (M − 15, 0.39), 151 (M − 29, 1.1), 138 (9.4), 137 (M − 43, 7.8), and 124 (M − 56, 100).

The butylene elimination is the main process (X*a*). If no other fragmentation reaction takes place in the cannabinoid molecule, this fragmentation should give the same formation rate and relative abundance of the M − 56 ion for all different kinds of cannabinoids.

McLafferty Rearrangement—This type of rearrangement is found for the derivatives of cannabinol (VI). Substituents in the aryl nucleus have no influence on the elimination of one of the geminal methyl groups (M − 15). The relative contribution of the fragmentation of the side chain in the total fragmentation process is strongly dependent upon the alicyclic structure of the cannabinoid. α-Carbon methyl substitution in the side chain favors the side-chain fragmentation, as evidenced by the relative abundances of the formation of M − 56 in various cannabinoids: I, 0; II, 5; III, 1; IV, 5; *trans-para-*V*a*, 45; *trans-para-*V*b*, 30; VI, 5; α-methyl-*trans-para-*V*a*, 80; α,α-dimethyl-*trans-para-*V*a*, 100; α-methylhexahydrocannabinol (VIII*b*), 100; and α,α-dimethylhexahydrocannabinol (VIII*c*), 100.

A fragmentation triggered only by the aryl nucleus also should rupture the substituted methyl group and, therefore, substantially increase the intensity of the M − 15 fragment (XI). This effect is not observed, so it must be concluded that the McLafferty rearrangement is favored. This proton transfer then is not sterically hindered by one or two substituted methyl groups. An increase in the fragmentation of the side chain can only be measured when the competing fragmentation reactions remain the same.





Scheme IV

Therefore, comparison of this fragmentation in all Δ^6-tetrahydrocannabinol derivatives is possible (Table IV). The side chain of *tert*-butyl-Δ^6-tetrahydrocannabinol is not fragmented at all, proving that the McLafferty rearrangement is the mechanism in the side-chain fragmentation. After the proton transfer, the carbon–carbon bond energy determines the fragmentation rate. There is no active participation of the alicyclic ring structure because saturation of the ring system does not alter the fragmentation pattern of the side chain (Table V).

Formation of Mass Fragment M − 68 (*m/e* 246)—Mass fragment *m/e* 246 is the result of a retro Diels–Alder reaction (Scheme IV). Loss of a geminal methyl group gives *m/e* 231, which has a pyran structure.

Is mass fragment *m/e* 246 a closed structure, as shown in Scheme IV, or an open structure resulting from the retro Diels–Alder reaction in, for instance, cannabidiol? With cannabidiol, the relative abundance of *m/e*

Table III—Formation of M − 43 in Different Isomers of Tetrahydrocannabinol (V) and Cannabidiol (IV) at 20 ev

Compound	Relative Intensity at 20 ev of *m/e* 271	*m/e* 285
Δ^6-*cis-ortho*	6	
Δ^6-*cis-para*	5	
Δ^6-*trans-ortho*	60	
Δ^6-*trans-para*	25	
Δ^6-*trans-para-O*-methyl		25
Δ^8-iso-*ortho*	12	
Δ^8-iso-*para*	10	
Δ^1-*cis-ortho*	100	
Δ^1-*cis-para*	100	
Δ^1-*trans-ortho*	32	
Δ^1-*trans-para*	30	
Δ^1-*trans-para-O*-methyl		20
IV	1	

Table IV—Influence of Substitution of the Side Chain on the Formation of M − 56 in Δ^6-*trans-para*-Tetrahydrocannabinol

Structure	C–C Bond	Relative Abundance of M − 56
-C-C-C-C-C	Secondary–secondary	45
-C(C)-C-C-C-C	Tertiary–secondary	80
-C(C)(C)-C-C-C-C	Quaternary–secondary	100
-C(C)(C)-C	No McLafferty rearrangement	1

Table V—Influence of the Alicyclic Ring in Isomers of Tetrahydrocannabinol (V) and Hexahydrocannabinol (VIII*a*) on the Fragmentation of the Side Chain at 20 ev

Compound	Relative Abundance of M − 56
Δ^6-*trans-para*-V	40
Δ^6-*trans-para*-α-methyl-V	32
Δ^1-*trans-para*-V	28
Δ^1-*trans-para*-α-methyl-V	25
Δ^6-*trans-ortho*-V	27
Δ^6-*trans-ortho*-α-methyl-V	21
Δ^1-*trans-ortho*-V	40
Δ^1-*trans-ortho*-methyl-V	38
trans-ortho-VIII*a*	42
trans-ortho-VIII*b*	49
trans-para-VIII*a*	75
trans-para-VIII*b*	50

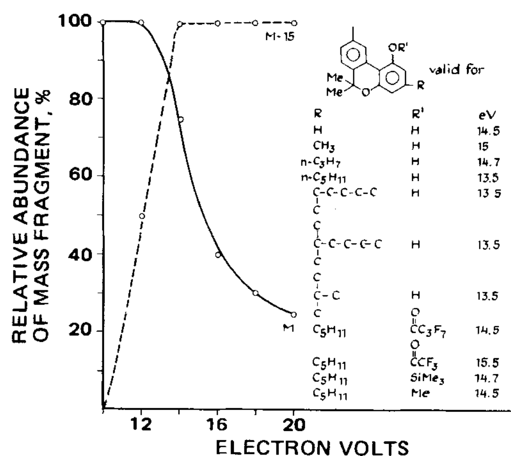


Figure 3—*Electron voltage mass fragmentograms of VI derivatives.*

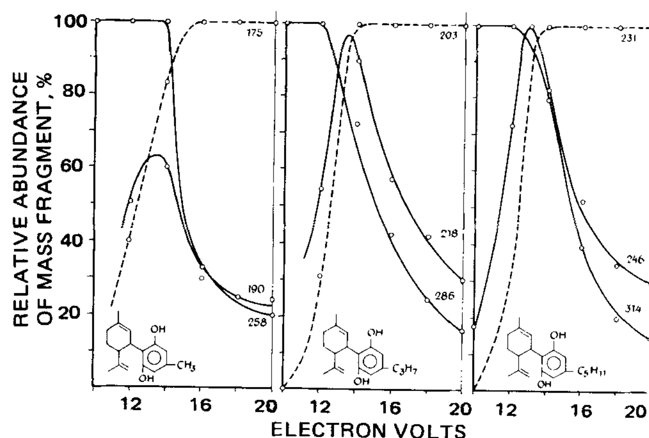


Figure 4—*Electron voltage mass fragmentograms of IV with different side chains occurring in natural hashish.*

246 shows a very characteristic rise and fall between 10 and 20 ev (Fig. 4), which is not seen in all other cannabinoids (6). The maximum relative abundance and, thus, the maximum concentration of the mass fragment occur at 14 ev. This finding may indicate that at the maximum the formation and fragmentation rates of *m/e* 246 are equal (Scheme V). In this case, ring closure must occur to make the loss of the geminal methyl group and the formation of *m/e* 231 possible.

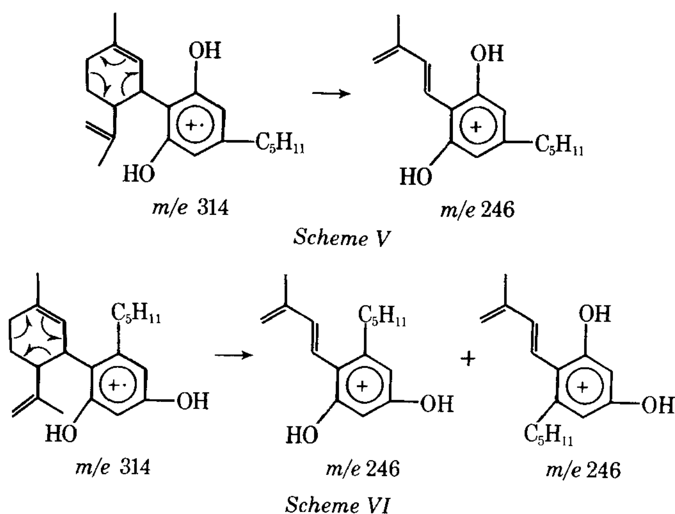
This ring closure, prior to the loss of the methyl group, can be rationalized as follows. In cannabidiol, two aromatic hydroxyl groups are available for ring closure. If, due to methylation or to the *ortho*-position of the side chain, only one or no hydroxyl groups is available, there is no ring closure, followed by the loss of a geminal methyl group (Scheme VI). If two hydroxyl groups are available for ring closure, the maximum of *m/e* 246 is sharp at 14 ev when the relative abundance of *m/e* 246 is plotted against the electron energy used (Fig. 4 and Table VI).

If only one hydroxyl group is available such as in *o*-cannabidiol (Scheme VI) and *p*-methoxycannabidiol (Table VI), there is a broad maximum of *m/e* 246 or 260 of about 2–3 ev. It takes more energy for ring closure. When no hydroxyl groups are available for ring closure, then *m/e* 246 reaches its maximum at 14 ev and remains the base peak until at least 20 ev.

Thus, it may be concluded that about 4 ev is required for the retro Diels–Alder reaction only and that 2–3 ev is required for rotation of the aromatic moiety around axis 3-3′ and ring closure. A similar figure is obtained in the loss of a geminal methyl group from cannabinol and cannabinodiol (3). There is a difference of 5 between the electron volts in which the relative abundances of *m/e* 310 and 295 (M − 15) are equal (Fig. 3). As explained in the section on M − 15, this energy is required for ring closure of cannabinodiol.

When the two hydroxyl groups of cannabidiol are methylated, there is still a relatively high relative abundance of *m/e* 231. To form *m/e* 231, a methyl must first be lost from the methoxy group, followed by ring closure and loss of a geminal methyl group.

Besides the retro Diels–Alder mechanism, a one-step mechanism for the formation of *m/e* 231 was postulated (5). With cannabidiol, there is perfect competition between these two mechanisms, with the retro Diels–Alder being favored at low electron energy (14 ev). The retro Diels–Alder mechanism is preferred if the hydroxyl groups are methylated, thus preventing attack of the phenolic hydroxyl group at the double bond, and if the electron energy is too low for the one-step mechanism (5).



Formation of Mass Fragment *m/e* 243 (M − 15 − 56)—Mass fragment *m/e* 243 is formed from: (*a*) loss of a geminal methyl group (M − 15) followed by loss of the side chain (M − 56), or (*b*) loss of the side chain (M − 56) followed by loss of the geminal methyl group. From these two reaction sequences, it follows that fragments *m/e* 299, 258, and 243 are dependent upon each other. When the relative abundances of these three fragments are added together, they represent about 38% of the total ion intensity of V*b* and its isomers and 18% of the total ion current of V*a* derivatives. The difference in contribution of these two sequential mechanisms between V*a* and V*b* is mainly due to the loss of the geminal methyl group (10% in V*a* and 60% in V*b*) and hardly to side-chain fragmentation, which is substantiated by the ratios of the relative intensities of M − 15 to M and the relative abundances of the formation of M − 56. It must be kept in mind that M − 15 is partly originated from the loss of the C-7 methyl group in V*a* (5).

Formation of Mass Fragment *m/e* 231—Mass fragment *m/e* 231 appears to be important in the mass spectra of cannabinoids. Its relative intensity varies between 30 and 100%, depending upon the structure of the compound. There are several mechanisms for the formation of *m/e* 231, including a simple cleavage in cannabichromene (Scheme VII). The relative intensity of *m/e* 231 in cannabichromene becomes 100% at 12 ev. The intensity of the molecular ion *m/e* 314 decreases so rapidly that the formation of *m/e* 231 must be a relatively easy process (5). All other fragments have a relative abundance of below 5%. This rapid formation

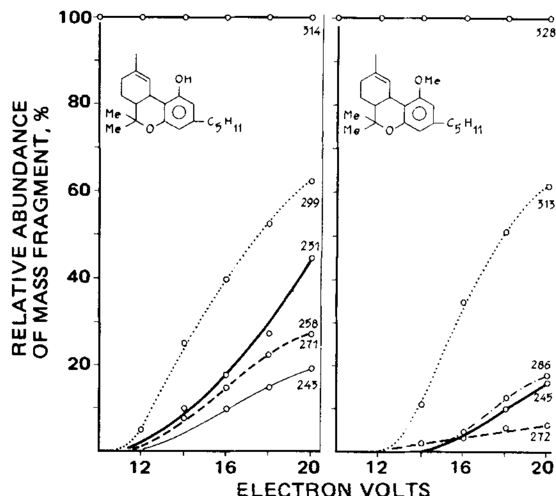


Figure 5—*Electron voltage mass fragmentograms of* V*b and its methyl ether. Note the difference in the formation rates of* m/e *231 and 245.*

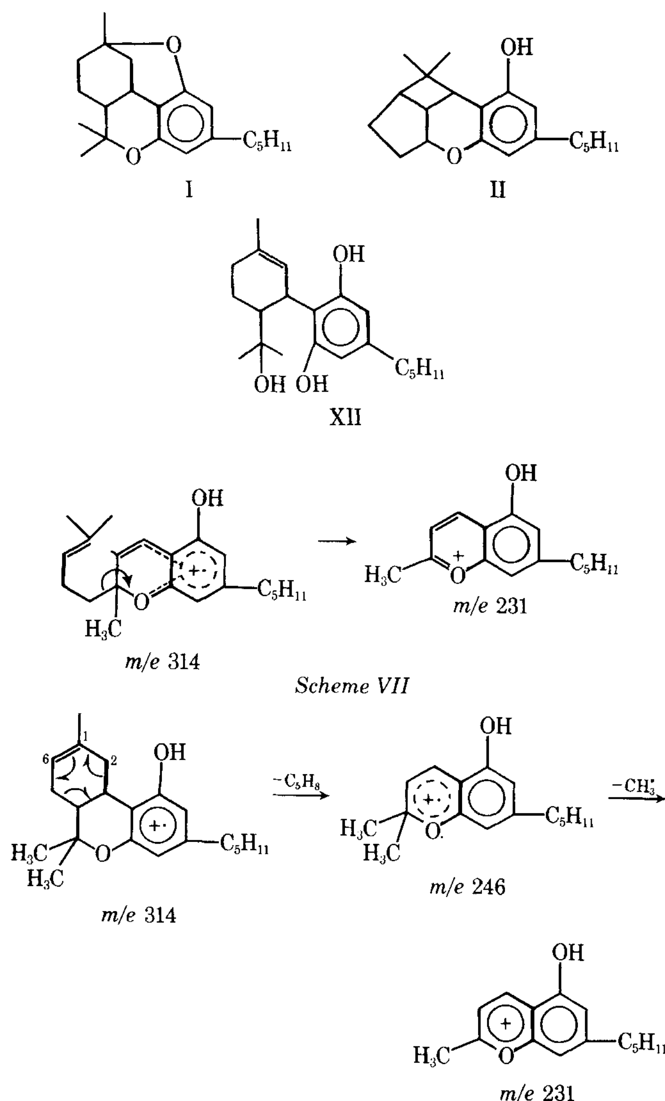
Table VI—Mass Spectra of Cannabidiol Derivatives at 20 ev

Structure	OH / HO / C_5H_{11}	C_5H_{11} / HO / OH	OCH_3 / HO / C_5H_{11}	C_5H_{11} / HO / OCH_3	OCH_3 / CH_3O / C_5H_{11}	C_5H_{11} / CH_3O / OCH_3	C_5H_{11} / CH_3O / OH
M	15	15	15	55	35	7	100
M − 15 (299)	1	1	1	15	5	5	75
M − 43 (271)	1	1	1	·10	5	5	20
M − 56 (258)	5	5	5	15	5	5	7
(246)	30	60	85	100	100	100	10
(243)	1	1	1	35	5	5	25
(231)	100	100	100	45	25	20	30
Maximum of *m/e* 246 at	14 ev	14–16 ev	13–16 ev	14–20 ev	14–20 ev	14–20 ev	No ev
Ratio of *m/e* 246/231	0.30	0.60	0.85	2.22	4.0	5.0	0.33

of *m/e* 231 also occurs, in folded structures such as cannabicitran (I), cannabicyclol (II), and 7-hydroxycannabidiol (XII).

With increasing power of separation of the cannabinoids, more and more naturally occurring cannabinoids are discovered that show mass spectral behavior as described. The mechanism of the formation of *m/e* 231 in these complicated structures is not yet clear.

Compound V*a* is fragmented to *m/e* 231 by means of a retro Diels–Alder reaction (*m/e* 246), followed by the loss of a geminal methyl group (Scheme VIII). There is no influence from the free hydroxyl group itself nor from the side chain or the methylated hydroxyl group (Table VII) (7). There is also a one-step mechanism in the formation of *m/e* 231, and



the two mechanisms are complementary (5).

In V*b*, a retro Diels–Alder reaction never results in the loss of fragment C_5H_8 and in *m/e* 246. Nevertheless, *m/e* 231 is found, so the delocalization of the 1–2-double bond (2, 4) must be possible, after which the one-step mechanism can take place.

The mass spectra of a series of derivatives and isomers of V*a* and V*b* reveal that more mechanisms are involved in the formation of *m/e* 231. Table VIII shows the mass spectra of *ortho*-V*b*, *para*-V*b*, and their methylated derivatives. Methylation of both *ortho*-V*b* and *para*-V*b* reveals that there is a sharp drop in the relative abundance of *m/e* 231 (*m/e* 245) in comparison with *ortho*- and *para*-V*b* (Fig. 5). This drop in intensity indicates that the phenolic proton is important for the generation of *m/e* 231 because it is not observed with V*a* and its methylated derivatives (7).

Strikingly, the mass spectrum of V*b*, deuterated at the phenolic hydroxyl group, shows complete retention of the deuterium label at the phenolic oxygen (5), in contradiction with the scheme given previously (7). This observation should mean that replacement of the phenolic hydrogen by a methyl group has no influence at the underlying mechanism of formation of mass fragment *m/e* 231. The mass spectra shown in Tables VII and VIII and those published elsewhere (5) are contradictory at first sight concerning the importance of the phenolic hydrogen. However, the retention of the phenolic proton observed in *m/e* 231 (5) and its influence on the formation of *m/e* 231 (7) can be rationalized satisfactorily by Scheme IX.

m/e 314

$-C_6H_{11}^{\cdot}$

m/e 231

Scheme IX

Table VII—Partial Mass Spectra of *trans-ortho-* and *trans-para-*Va and Their Methyl Ethers at 20 ev

Fragment	m/e	ortho-Va	ortho-Methyl-Va	para-Va	para-Methyl-Va
M	314 (328)	100	100	100	100
M − 15	299 (313)	15	19	13	13
M − 43	271 (285)	60	70	25	22
M − 56	258 (272)	27	21	40	32
M − 68	246 (260)	10	5	17	22
	243 (257)	2	1	5	5
	231 (245)	65	60	60	75

Table VIII—Partial Mass Spectra of *trans-ortho-* and *trans-para-*Vb and Their Methyl Ethers at 20 ev

Fragment	m/e	ortho-Vb	ortho-Methyl-Vb	para-Vb	para-Methyl-Vb
M	314 (328)	100	100	100	100
M − 15	299 (313)	55	77	63	62
M − 43	271 (285)	32	42	30	17
M − 56	258 (272)	25	31	27	26
M − 68	246 (260)	10	3	5	2
	243 (257)	10	5	20	5
	231 (245)	72	15	40	16

Table IX—Relative Abundance of *m/e* 231 (*m/e* 245) in the Mass Spectra of Several Tetrahydrocannabinol (V) Isomers Taken at 20 ev

Compound	Relative Abundance	Mechanism
*cis-ortho-*Vb	11	Isomerization
*cis-para-*Vb	12	Isomerization
*trans-ortho-O-*methyl-Vb	15	Isomerization
*trans-para-O-*methyl-Vb	16	Isomerization
*trans-ortho-*Vb	72	Internal proton transfer plus isomerization
*trans-para-*Vb	40	Internal proton transfer plus isomerization minus bond formation
*cis-ortho-*Va	100	Retro Diels–Alder
*cis-para-*Va	100	Retro Diels–Alder
*trans-ortho-*Va	65	Retro Diels–Alder
*trans-ortho-O-*methyl-Va	60	Retro Diels–Alder
*trans-para-*Va	60	Retro Diels–Alder
*trans-para-O-*methyl-Va	75	Retro Diels–Alder

The fragmentation of cannabinoids is strongly dependent upon ion source temperature (5) and electron voltage (6). Results obtained with, for instance, 10–20 (250°) and 70 (120°) ev may reveal a complete shift in the relative contribution of complementary mechanisms that are possible for the formation of *m/e* 231. Methylation of both *ortho-* and *para-*isomers of Vb does not inhibit the formation of *m/e* 245 (*m/e* 231) completely. Since the relative abundance of *m/e* 245 is 15–16% in both isomers, the mechanism underlying this fragmentation must be the same: isomerization of the 1-2-double bond (Δ^1) to the 1-6-position (Δ^6) as suggested earlier (4) and visualized in Scheme X.

From the intensities of *m/e* 231 and 245, the spectra of *trans-ortho-*Vb and its methyl ether (Table VIII), it can be calculated that about 80% of *m/e* 231 is generated by internal protonation at 20 ev and that the contribution of the isomerization of the double bond is 20%, assuming that

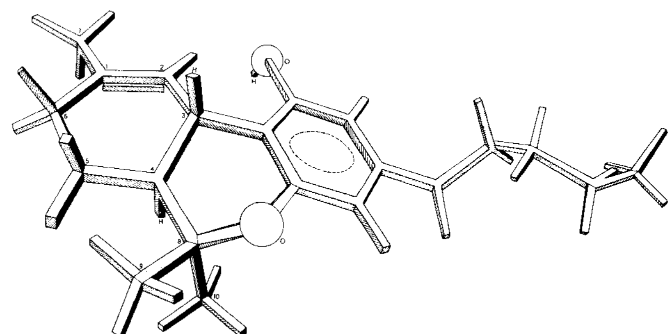
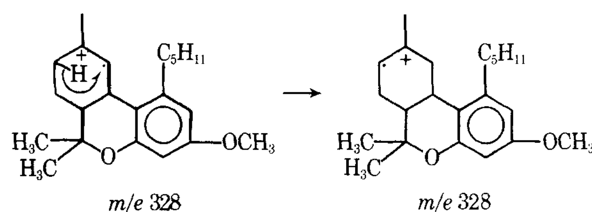
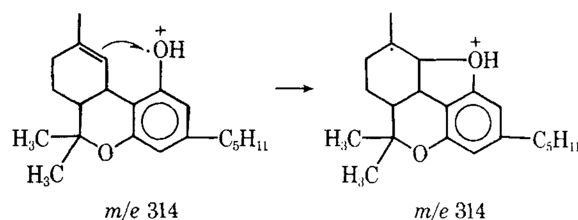


Figure 6—*Molecular structure of* trans-para-*V*b.



Scheme X



Scheme XI

the latter is not influenced by the methylation. After correction for the contribution of the isomerization (15%), the relative abundances of *m/e* 231 from *trans-ortho-* and *trans-para-*Vb appear to be 57 and 25%, respectively. This difference is not observed for the relative intensities of *trans-ortho-* and *trans-para-*Va (Table VII).

This result implies that the internal protonation must be reduced, presumably due to the position of the phenolic hydroxyl group, because methylation does not influence the isomerization of the 1-2-double bond. A possible explanation is that the phenolic oxygen atom in *trans-para-*Vb attacks the double bond, generating an ion with four fused rings. Whether this process occurs before or after phenolic proton transfer is not certain.

This bond destroys the 1-2-double bond, so that the phenolic proton can no longer be transferred to the alicyclic ring. This bond formation is impossible in *trans-ortho-*Vb and *cis-para-* and *cis-ortho-*Vb (11 and 12% of *m/e* 231, respectively) (Scheme XI). Therefore, in the formation of *m/e* 231 from *trans-para-*Vb, three fragmentation pathways may be distinguished (at 20 ev): 35% internal protonation (plus a one-step mechanism), 20% isomerization of the 1-2-double bond (retro Diels–Alder plus a one-step mechanism), and 45% bond formation.

***cis–trans*-Isomers of *ortho-* and *para-*Vb**—In *cis-*Va and *cis-*Vb, the alicyclic ring system is perpendicular to the aromatic system (Fig. 7, compare Fig. 6). Therefore, in the formation of *m/e* 231, there are no bond formation and internal shift. The relative abundances of *m/e* 231 derived from *cis-ortho-* and *cis-para-*Vb are equal to those derived from the methyl ethers of *trans-ortho-* and *trans-para-*Vb (Table IX).

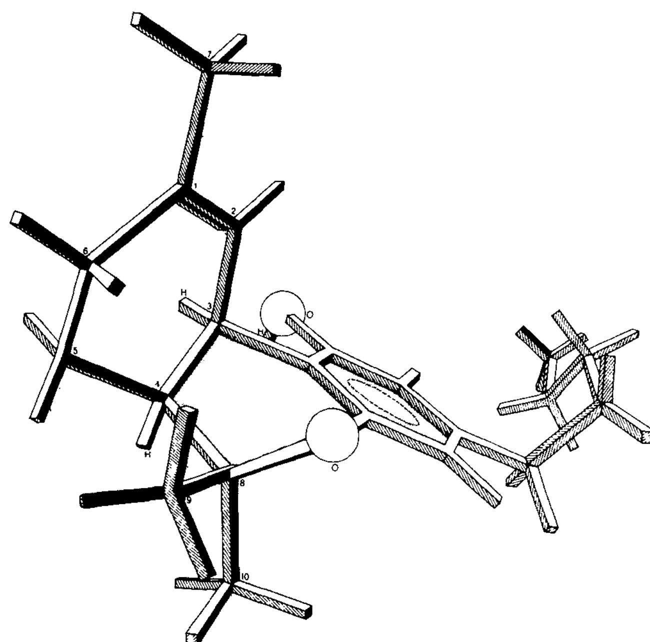


Figure 7—*Molecular structure of* cis-para-*V*b.

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Chronotropic and Cyclic Adenosine Monophosphate Response of Fetal Rat Heart in Organ Culture to Isoproterenol, Quinidine, and a Dysrhythmogenic Agent

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Abstract ☐ The fetal rat heart in organ culture was used to investigate rate changes by various cardioactive agents. Concomitantly, the effect of these pharmacologically induced rate changes on steady-state cyclic adenosine monophosphate levels was determined. Isoproterenol increased the fetal rat heart rate and cyclic adenosine monophosphate in a concentration-related fashion. Quinidine produced a concentration-related decrease in heart rate and no change in cyclic adenosine monophosphate level. The dysrhythmogenic agent produced concentration-related negative chronotropism in the fetal rat heart preparation and significant elevations in cyclic adenosine monophosphate at concentrations without chronotropic action. No correlation between chronotropic effect of a drug and cyclic adenosine monophosphate levels was observed.

Keyphrases ☐ Isoproterenol—effect on fetal rat heart rate related to levels of cyclic adenosine monophosphate, organ culture ☐ Quinidine—effect on fetal rat heart rate related to levels of cyclic adenosine monophosphate, organ culture ☐ Dysrhythmogenic agent—effect on fetal rat heart rate related to levels of cyclic adenosine monophosphate, organ culture ☐ Adenosine monophosphate, cyclic—levels in fetal rat heart in culture, related to rate changes caused by isoproterenol, quinidine, and dysrhythmogenic agent ☐ Cardioactive agents—isoproterenol, quinidine, and dysrhythmogenic agent, effect on fetal rat heart rate related to levels of cyclic adenosine monophosphate, organ culture ☐ Chronotropic effects—isoproterenol, quinidine, and dysrhythmogenic agent, fetal rat heart in culture, related to levels of cyclic adenosine monophosphate

Although the role of cyclic adenosine monophosphate (I) in cardiac inotropism has been investigated (1–3), there have been few attempts to study its role in cardiac chronotropism. The pacemaker region of the heart has been considered too small to be of use in determining adenyl cyclase activity, phosphodiesterase activity, or I levels (4). One study (5) demonstrated that chronotropic and inotropic cardiac responses to catecholamines were very similar with respect to dose dependence and sensitivity to β-blocking agents. This result might be considered as indirect evidence of a common mechanism for cardiac rate and force of contraction. Much evidence linking chronotropism and I comes from the study of cultured, beating heart cells isolated from the neonatal rat. The pulsation rate of these cells is accelerated by epinephrine, a known stimulator of adenyl cyclase (6). Dibutyryl I also imposes positive chronotropism in the same preparation (7).

Entire hearts from fetal mice can be cultured in such a manner as to beat consistently for a period of time (8). The cultured fetal mouse heart has been used as a pharmacological tool. Positive chronotropism was demonstrated using this preparation with liothyronine and levarterenol, and negative chronotropism was found with acetylcholine (9). The fetal mouse heart is capable of responding to these agents as early as the 12th day of gestation (10). The presence of the β-receptor was demonstrated in this same preparation (11).

In this study, the entire fetal rat heart was cultured and used to investigate the relationship between chronotropic effects and steady-state I levels in the myocardium. The fetal rat heart was chosen because it was larger than the mouse heart; this larger size facilitated tissue assay.

Three agents were chosen for pharmacological intervention. Isoproterenol was used for its positive chronotropic and β-adrenergic actions. Quinidine was employed for its negative chronotropic effect. Ethyl 3-ethoxycarbonyl-4-hydroxy-2*H*-1,2-benzothiazine-2-acetate 1,1-dioxide[1] (II), an experimental compound that produces ventricular fibrillation (12), was used to investigate the role of I in cardiac dysrhythmogenesis.

EXPERIMENTAL

Organ Culture—On their 19th day of gestation, pregnant Sprague–Dawley rats were sacrificed by cervical separation. The fetuses were quickly removed by Caesarean section.

The fetuses were decapitated, the chests were cut open, and the fetal hearts were removed as quickly and aseptically as possible. The hearts were trimmed of excessive connective tissue, rinsed in sterile saline, and

[1] McN-2165, McNeil Laboratories.