Tetrahedron. Vol. 30, pp. 4243 to 4248. Pergamon Press 1974. Printed in Great Britain

THE USE OF METASTABLE ION CHARACTERISTICS FOR THE DETERMINATION OF ION STRUCTURES OF SOME ISOMERIC CANNABINOIDS

J. K. TERLOUW, W. HEERMA*, P. C. BURGERS and G. DIJKSTRA
Laboratory for Analytical Chemistry, University of Utrecht, Croesestraat 77A, The Netherlands

and

A. BOON, H. F. KRAMER and C. A. SALEMINK
Laboratory for Organic Chemistry, University of Utrecht, Croesestraat 79, The Netherlands

(*Received in the UK* 1 *July* 1974; *Accepted for publication* 12 *July* 1974)

Abstract—The mechanism of formation of the prominent $C_{15}H_{19}O_2$ ion at *m/e* 231 in the mass spectra of $\Delta^{1(6)}$-tetrahydrocannabinol and five isomeric cannabinoids has been investigated. Except via a well-documented two step process, involving an RDA mechanism, a sizeable percentage of these ions is formed by a novel one-step route from the molecular ion. This was deduced from the spectra of deuterium-labelled compounds and measurements of the kinetic energy release of metastable ions. The latter value for the one step process varies from 25–44 meV for the six compounds investigated, attributed to two interdependent effects, different transition state geometries and common transition states differing in the time elapsing before their formation.

The interest in the chemical and pharmacological properties of hashish, the well-known cannabis resin, is still current. Progress in this field has been rapid and mass spectrometric information has considerably aided in the structure elucidation of many major components.

The analysis of minor components and metabolites, which are usually available only in small quantities, will undoubtedly require that most of the structural information has to be derived from the mass spectrum.

It should be noted, however, that gross structural differences in the terpene moiety of known cannabinoids are reflected in their mass spectra only by different abundances of ions with the same elemental composition. This is illustrated in Table 1, where the intensities of the principal ions of a number of cannabinoids of molecular weight 314 are listed.† Fragmentation mechanisms of some of these compounds have been postulated,[1-4] based upon more or less obvious fragmentation rules and a few experiments with labelled compounds, but they are still partly obscure.

Structural analysis of metabolites will be facilitated by a better understanding of the fragmentation behaviour of these compounds. Moreover, the similarity of the spectra makes it important for direct analysis to have criteria for characterizing the *ion structures*. With regard to the latter, a fruitful approach might be found in the kinetic energy (T) released during the fragmentation of metastable ions.

†In order to maintain a uniform indication of corresponding positions in the different compounds the monoterpene numbering has been used in this paper.

Cooks and Beynon[5] have pointed out that identical or nearly identical values of T should correspond with identical ion structures of ions formed from different sources.

As we are dealing with metastable ions, i.e. ions with a closely fixed excess internal energy, we have the advantage that T is relatively insensitive to variations in internal energy.

As a consequence, the information obtained should be applied with care to the structure of both the non fragmenting ions and the ions dissociating in the ion source.

In this paper some preliminary findings on the major fragmentation behaviour of these cannabinoids, based on both T-values and isotopic labelling, are discussed.

RESULTS AND DISCUSSION

The reaction studied is the formation of the common $C_{15}H_{19}O_2$ ion at *m/e* 231. Metastable measurements by the defocusing technique indicated that this ion can be produced by the pathways shown in Figure 1.

Formation of the *m/e* 231 ions via that of *m/e* 299 occurs only to a minor extent, as is indicated by the metastable intensities, which are an order of magnitude less than those for the other two processes. Their relative metastable intensities (the ratio metastable/daughter ion) vary considerably with molecular structure. This is shown in Table 2.

Although these figures should be interpreted with care, they do suggest that the first three compounds of Table 2

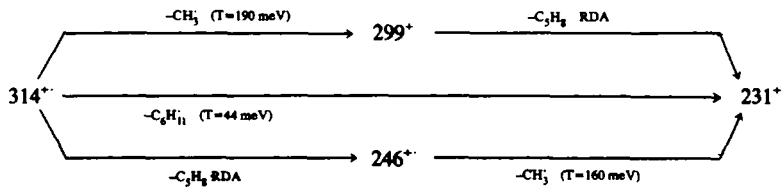
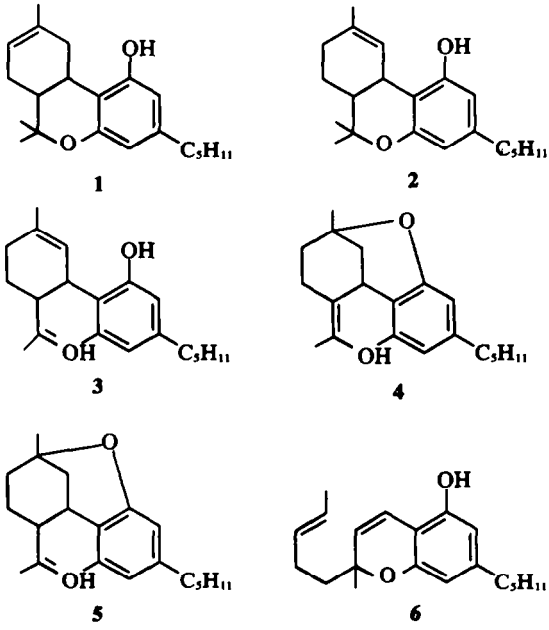


Fig 1. Fragmentation pathways leading to the formation of *m/e* 231 ions in M.W. 314 cannabinoids. The T-values are for 1,6-THC.

Table 1. Intensities of the principal ions of six M.W. 314 cannabinoids



1 = $\Delta^{1(6)}$-trans-tetrahydrocannabinol (1,6-THC)
2 = $\Delta^{1(2)}$-trans-tetrahydrocannabinol (1,2-THC)
3 = $\Delta^{1(2)}$-trans-cannabidiol (CBD)
4 = $\Delta^{4(8)}$-iso-tetrahydrocannabinol (4,8-iso-THC)
5 = $\Delta^{8(9)}$-iso-tetrahydrocannabinol (8,9-iso-THC)
6 = cannabichromene (CBC)

	Intensity (% of base peak)					
m/e	1	2	3	4	5	6
314	100	100	30	100	31	9
299	6	51	3	28	6	5
271	28	22	2	83	5	1
258	32	15	2	39	5	1
246	16	4	47	11	2	
231	66	32	100	60	100	100
193	17	5	14	19	4	1
174	8	4	9	6	11	11

fragment preferentially via the 246 ion. This reaction has been explained by Budzikiewicz *et al*[1] and Claussen *et al*[2] as a well-known retro Diels–Alder (RDA) reaction (elimination of a C_5H_8 neutral fragment), followed by loss of a CH_3 radical (see Figure 3). In the 1,2-isomer this reaction is thought to occur after isomerisation of the double bond to the 1,6 position.[1,4]

Table 2. Metastable/daughter ion intensity ratios for the transitions 314 → 231 and 246 → 231

Compound	$\frac{I\ m^*\ 314 \rightarrow 231}{I\ 231}$ (%)	$\frac{I\ m^*\ 246 \rightarrow 231}{I\ 231}$ (%)
1,6-THC	0·8	2·3
1,2-THC	1·0	3·8
CBD	0·2	12·5
4,8-iso-THC	2·3	0·4
8,9-iso-THC	3·8	0·8
CBC	0·4	0·03

Nevertheless, *m/e* 231 ions can also be formed *directly* from the molecular ions; for the two iso-THC compounds and cannabichromene it is probable that this is even the main fragmentation pathway.

Especially in the case of the THC's and CBD, however, the metastable transitions observed for the direct formation reaction might be due to a consecutive reaction. Therefore, T-values were measured for both the direct reaction and the "246" pathway. Some results are tabulated in Table 3.

From these results it can be concluded that the direct reaction does not consist of consecutive reactions via the "246" pathway.[6] Therefore, there must be another way of formation of the *m/e* 231 ions. The question then arises whether the reaction mechanism for this direct reaction is similar for all six compounds investigated.

The reaction is characterized by a relatively small T-value, which means that the kinetic energy release is primarily determined by the excess energy of the transition state complex.[5] This being the case, the slightly different T-values (Table 3) can be the result of either different transition state geometries or a unique transition state whose energy population is kinetically controlled (vide infra).

On the other hand the reaction $246^{+\cdot} \rightarrow 231^+$ has a large value of T. This involves a reaction which is mainly determined by a reverse activation energy.[5] Differences in T-values for this type of reaction are a much clearer criterion for differences in ion structure. The difference for this T-value between both THC compounds and CBD can be rationalized by assuming different structures for the fragmenting *m/e* 246 ions as shown in Figure 3.

In order to obtain more insight into the mechanism of the direct reaction and to estimate its relative importance, we have investigated the mass spectra of two deuterium-labelled 1,6-THC compounds, 3-deutero-1,6-THC and 7,7,7-trideutero-1,6-THC. Some representative mass spectra, recorded at two different ion source temperatures

Table 3. T-values (meV) corresponding with the metastable formation and degradation of the *m/e* 231 ion in some cannabinoids

	1,6-THC	1,2-THC	CBD	4,8-iso-THC	8,9-iso-THC	CBC
T_{231}^{314}	44	39	48	34	28	25
T_{231}^{246}	160	160	270	180[a]		
T_{174}^{231}	94	97	92	99	94	91

[a] This value is less reliable due to its small intensity. All other values, which are calculated from the half-width of the metastable, were reproducible to within 10%.

are presented in Figure 2a-d, together with the spectrum of the OD labelled 7,7,7-trideutero-1,6-THC compound, which is presented in Figure 2e.

As can be seen in Figure 2 the relative intensity distribution appears to be influenced by the ion-source temperature, but it is clear that in the case of the 3-D labelled compound the label is completely retained in all important fragment ions. This indicates that the mechanism of the formation of the *m/e* 271 ions proposed by Budzikiewicz *et al*[1] is not correct.

In the spectrum of the CD_3-labelled compound two striking features can be observed: (a) The vinylic methyl group at carbon atom 1 is eliminated, and is so to the same extent as the well documented loss of one of the gem-methyl groups; (b) A considerable fraction of the ions at *m/e* 231 is shifted to *m/e* 234, indicating the presence of the vinylic methyl group in this fragment ion. The latter fragmentation has been proved to be identical with the direct reaction leading to the production of the *m/e* 231 ion in the unlabelled compound. A metastable transition has been found for the reaction $317^{+\cdot} \rightarrow 234^{+}$, while the *m/e* 231 ions in this compound were found to be formed from the *m/e* 246 ions. From these data it follows that for the 1,6-THC compound, which is a priori expected to form *m/e* 231 ions mainly via the RDA mechanism, about 33% of these ions are generated by the distinct one step mechanism. Furthermore AP measurements on both the *m/e* 234 and *m/e* 231 ions showed that the direct reaction is energetically more favourable (12·2 eV as compared with 12·7 eV). All these observations suggest the mechanism presented in Figure 3 for the formation of these two ions.

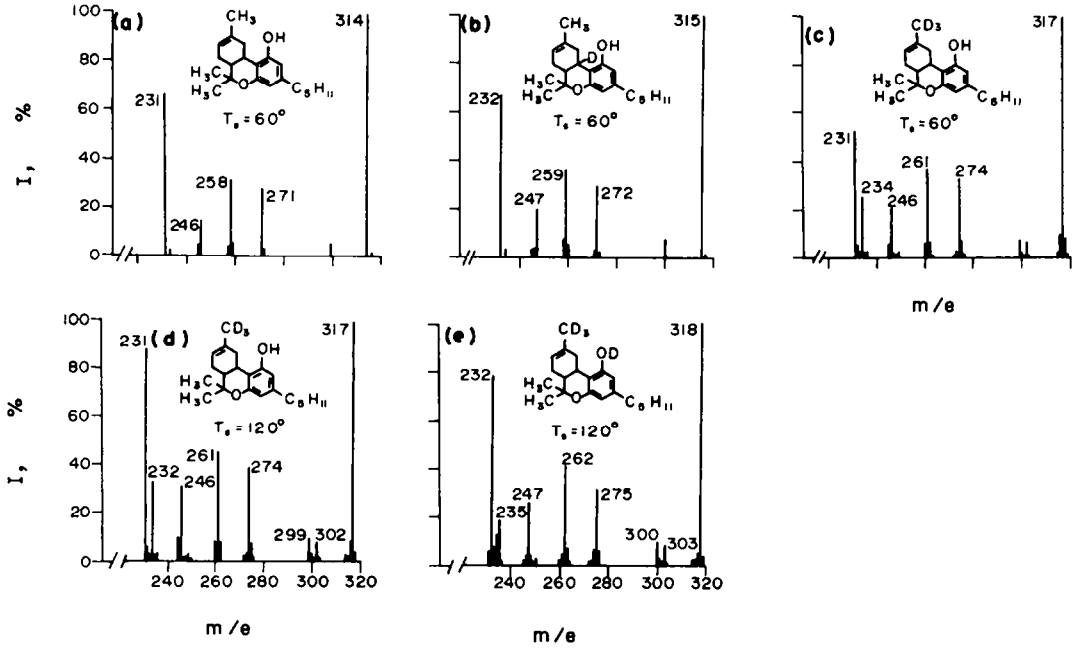


Fig 2. Partial mass spectra of 3,4-*trans*-$\Delta^{1(6)}$-THC. (a) the unlabelled compound recorded at an ion source temperature of 60°C; (b) the 3-D labelled compound at 60°C; (c) the 7,7,7-trideutero compound at 60°C; (d) the 7,7,7-trideutero compound at 120°C; and (e) the 7,7,7-trideutero compound in which the phenolic hydrogen atom has also been replaced by deuterium at 120°C. All spectra have been corrected for 13-C contributions, the O-D compound has also been corrected for incomplete labelling

The ionized vinylic double bond induces a β-hydrogen shift and fission of the C(8)–O bond resulting in the intermediate (β), followed by ring closure. The intermediate (δ) generated along these lines can easily lose the CD_3 or the C_6H_{11} radical resulting in the formation of the stable even-electron ions with m/e 299 and m/e 234. The β-hydrogen shift in intermediate (α), however, has to compete with a transfer of the phenolic hydrogen atom presumably to the C-6 position. This again results in the formation of m/e 234 ions, after rearrangement and elimination of C_6H_{11} radicals. This alternative pathway became obvious when studying the spectrum of the CD_3-OD labelled compound in which a substantial amount of the expected m/e 235 ion is shifted to m/e 234 (see Figure 2e).

It is worth noting that the structure of the ion formed by the direct reaction ($m/e = 234$) is identical to that of the m/e 231 ion formed via the two-step process.

The results obtained for 1,6-THC lead to the following expectations for the production of m/e 231 ions in the 1,2-isomer: (a) In comparison with 1,6-THC the direct production of m/e 231 ions more effectively competes with the two-step process. The latter pathway now requires an extra isomerisation (1,2→1,6) of the double bond, while the intermediate (δ) (see Figure 3) in the direct reaction can be generated without the hydrogen transfer mentioned; (b) Transfer of the phenolic hydrogen to a position in the neutral moiety lost in the 314→231 fragmentation does not occur.

Examination of the spectra of both 7,7,7-trideutero-1,2-THC as well as 1,2-THC OD proved that 62%* of the m/e 231 ions is formed in the direct reaction, while the spectrum of the OD compound revealed the expected complete retention of label.†

Furthermore, these observations rule out the alternative fragmentation pathway for the formation of m/e 231 ions proposed by Vree and Nibbering.[4] These authors propose the fragmentation of *trans*-para- and *trans*-ortho-1,2-THC mainly to occur after transfer of the phenolic proton via the π-orbital of the aromatic ring to position 2 in the terpene moiety. In that mechanism, the phenolic label should be lost in the m/e 231 ion formation, which is in contradiction with our observations. The differences in the mass spectra of 1,2-THC, 1,6-THC and their corresponding mono-methyl ethers, which led these authors to that proposal, can also be explained by our direct mechanism.

As has been stated before, the direct reaction also occurs in the other compounds not yet discussed. Plausible fragmentation pathways for these compounds (CBC, CBD and the two iso-THC's) are presented in Figure 3. Probably in all compounds the structure of the m/e 231 ion formed is identical.‡ This is supported by the observation that both the m/e 231/m/e 174 ion intensity ratio is consistent and the fact that equal T-values are found (see Table 3) for the metastable formation of m/e 174 ions (elimination of a butyl radical from the side chain).

On the other hand the transition state geometries of the fragmenting 314→231 ions are probably not identical for all six compounds. The reaction mechanisms postulated in Figure 3 being correct, three distinct activated complexes exist *viz.* (1) structure (δ) which CBC, 1,2-THC and 1,6-THC have in common, (2) structure (β') for both CBD and 8,9-iso-THC and (3) structure (β'') for 4,8-iso-THC.§

Although CBC, 1,2-THC and 1,6-THC are supposed to fragment via a unique transition state, the T-values found (Table 3) for this reaction are not the same: CBC having a significantly smaller value than the two THC-isomers.

This disparity can be associated with the time interval necessary for generation of the transition state: CBC can attain its transition state geometry much earlier than the two THC compounds, in which several intermediate steps are involved. As a result the average internal energy and the corresponding T-value of the precursor ions fragmenting in the first field free region is expected to be smaller for CBC than for the two THC isomers.¶ The same trend is observed in the case of CBD and 8,9-iso-THC. Again a common transition state is involved but the T-values differ, in agreement with the differing time intervals for transition state formation.

The variation in T-values observed for the reactions associated with a unique transition state but a different internal energy content are of such an order of magnitude that an overlap occurs with the T-values associated with different transition states.

As a consequence we feel that small T-values (corresponding with reactions mainly determined by the excess energy of the activated complex) should be interpreted with care.

EXPERIMENTAL

All mass spectrometric measurements were performed on an AEI MS 902 or MS 9 mass spectrometer, nominally operating at 70 eV electron energy and 50–70°C or 120°C ion chamber temperature. Samples were introduced via the direct inlet system. Elemental compositions were derived from element lists obtained by on line measurements of exact masses with an AEI MS 902 Argus 500 computer combination at a dynamic resolving power of 10,000. Metastable transitions have been traced by the defocusing technique of Jennings; T-measurements were performed on a modified MS-9 instrument, provided with a variable monitor electrode, using the HV-scan method. Labelling of the phenolic hydrogen was performed by introducing the unlabelled compound in an ion source, in which a partial D_2O pressure of 3×10^{-6} Torr

*7,7,7-trideutero-1,2-THC showed the following mass spectrum (% of base peak): 317(100), 302(15), 299(34), 274(29), 261(21), 246(17), 234(23), 231(14), 193(12), 177(4), 174(1).

†In the partially OD labelled compound metastable transitions could be observed for the reactions 246→231 and 247→232 but not for 247→231.

‡The structures of the accompanying C_xH_y neutrals are probably not identical.

§The proposed transition state (β'') also accounts for the high intensity of the M-C_3H_7 ions at m/e 271 (see Table 1).

¶An analogous explanation has been given by Cooks *et al*[5] for the differences in T-values observed between reactions in which identical ion structures were generated by on the one side direct ionization and on the other side an intermediate fragmentation step (see also E. G. Jones, L. E. Bauman, J. H. Beynon and R. G. Cooks, *Org. Mass Spectrom.* 7, 185 (1973)).

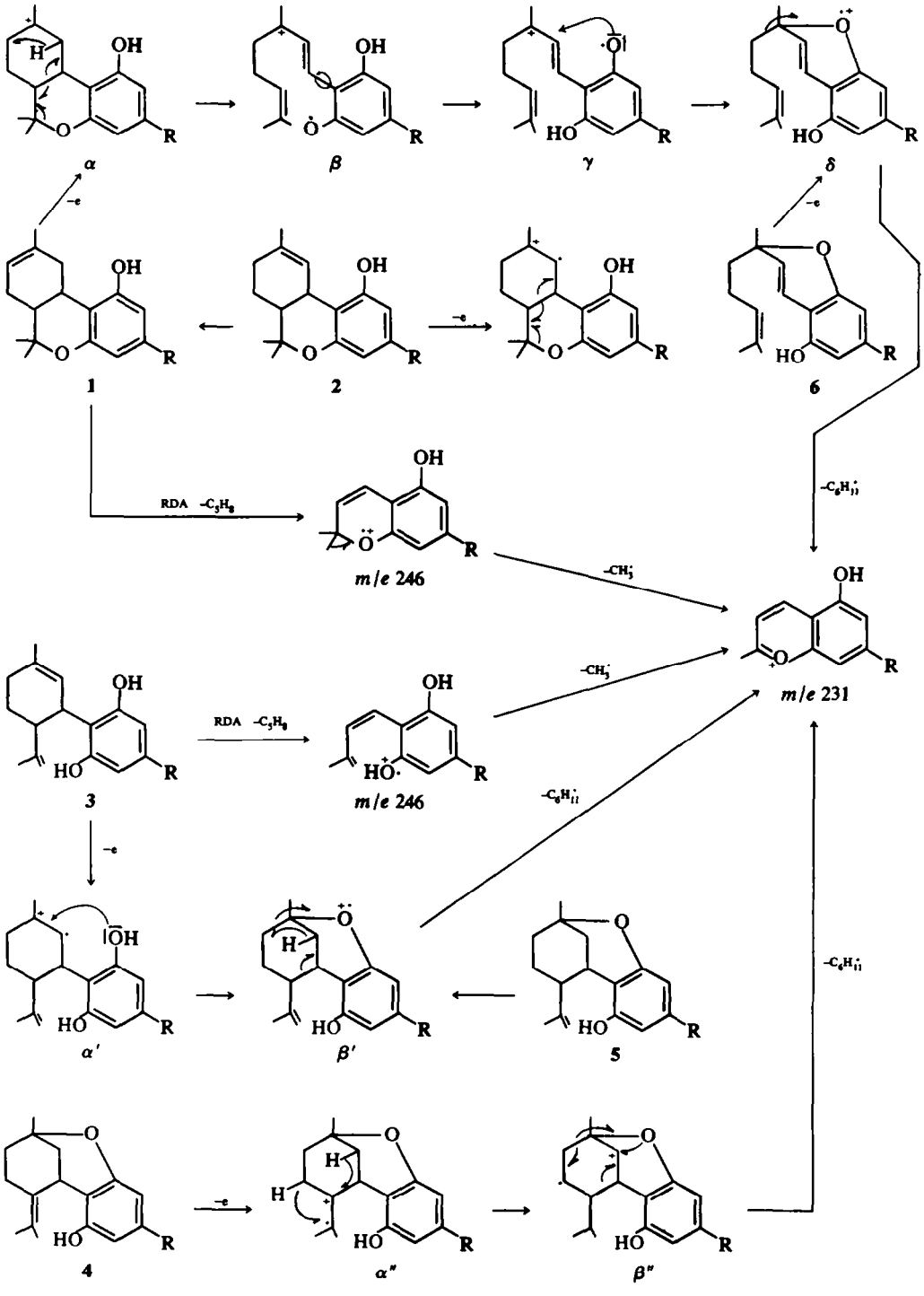


Fig 3. Postulated fragmentation mechanisms for the formation of *m/e* 231 ions in 1,6-THC (1), 1,2-THC (2), CBD (3), 4,8-iso-THC (4), 8,9-iso-THC (5) and CBC (6)

was maintained at 60°C. The purity of all unlabelled compounds, which have been isolated or synthesized by known procedures, was checked by measurements on a Jeol JMS-07 GC-MS system.

3-Deutero-$\Delta^{1(6)}$-THC was synthesized by the method described by Mechoulam *et al.*[7] 7,7,7-Trideutero-$\Delta^{1(6)}$-THC was prepared analogous to the method of Fahrenholtz *et al*[8] using CD_3MgI. 7,7,7-Trideutero $\Delta^{1(2)}$-THC was prepared via the method described by Petrzilka *et al*[9] starting from 7,7,7-trideutero-$\Delta^{1(6)}$-THC.

Acknowledgements—We wish to thank the National Department of Public Health and Environmental Hygienes for financial support. We also wish to thank Prof. J. L. Holmes (University of Ottawa, Canada) and Prof. R. Mechoulam (Hebrew University, Jerusalem) for valuable discussions, Ir. J. G. van Heyst for providing the instrumental facilities and Dr. F. J. E. M. Küppers for synthetical work.

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