Mass Spectra of Some Diterpenoids with the Novel Carbon Skeletons Verrucosane, Neoverrucosane and Homoverrucosane

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〔質量分析, Vol. 30, No. 4, p. 325～335 (1982), 別刷〕

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(Received September 17, 1982)

The mass spectra of fifteen diterpenoids belonging to the new carbon skeletons of verrucosane, neoverrucosane and homoverrucosane are examined. From their spectra the relationship between fragmentation patterns and substituted modes of these diterpenoids is discussed.

1. Introduction

In the course of our study on terpenoids of the liverworts, we have isolated several new diterpenoids from an ethanolic extract of a leafy liverwort *Mylia verrucosa* Lindb. Their structures including the absolute configurations have been determined on th basis of the results of the extensive chemical reactions and X-ray analysis to have the novel carbon skeletons named "verrucosane" and "neoverrucosane" which are consisting of 3,6,6,5-tetracyclic carbon skeleton with *cis-trans-anti-trans*-ring juncture system.[1–5] The third novel carbon skeleton "homoverrucosane" of 7,6,5-tricyclic framework with *trans-anti-trans*-system has been obtained by ring opening reaction of the natural cyclopropyl carbinols.[1,5] The numbering of carbon atoms in three novel diterpene skeletons has been suggested in the original papers.[1–5]

In this paper we describe the electron impact induced fragmentations and the mass spectroscopic features of fifteen diterpenoids having the novel verrucosane, neoverrucosane and/or homoverrucosane skeletons. Of these compounds, the seven

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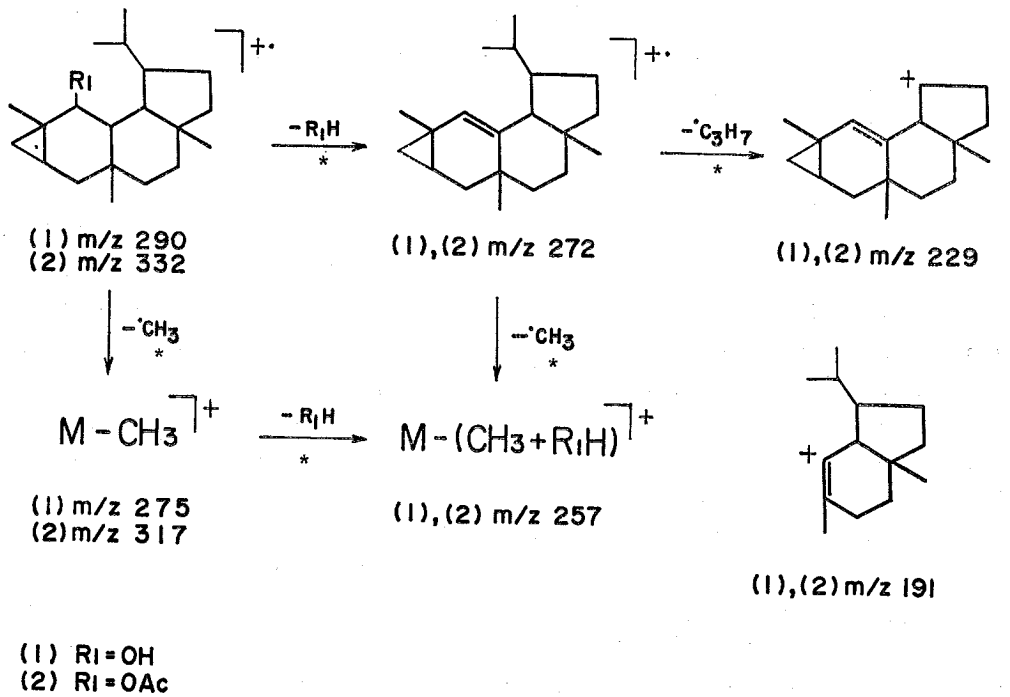
diterpenoids, 2β-hydroxy- (**1**), 2β,9α-dihydroxy- (**4**), 9α-acetoxy-2β-hydroxy- (**5**), 2β-hydroxy-9-oxo- (**12**), 11α-acetoxy-2β-hydroxy- (**7**), 2β-acetoxy-11α-hydroxyverrucosane (**8**) and 5β-hydroxyneoverrucosane (**14**) have been isolated as the natural products. Other eight, 2β-acetoxy- (**2**), 2β,11α-dihydroxy- (**6**), 2β,11α-diacetoxy- (**9**), 2β-acetoxy-11-oxoverrucosane (**13**), 5β-hydroxy- (**3**), 5β,11α-dihydroxy- (**10**), 2β-hydroxy-11α-acetoxyhomoverrucosane (**11**), 5β-acetoxyneoverrucosane (**15**) have been produced by chemical treatment of the natural products.

2. Results and Discussion

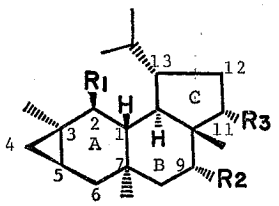
Mass spectra of 2β-hydroxyverrucosane (1) and 2β-acetoxyverrucosane (2).

2β-Hydroxyverrucosane (**1**) was isolated as a possible precursor into the two oxygen-functionallized verrucosane diterpenoids from the ethanol extract and its spectrum was examined in the first. Mass spectra of the verrucosanol (**1**) and its acetate (**2**) shown, respectively, in Fig. 1 and Table I exhibited similar fragmentation pathways which could be explained as depicted in Scheme 1.

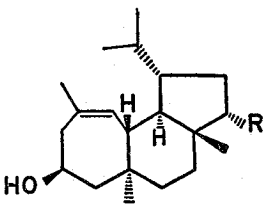


Scheme 1.

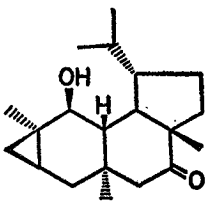
The loss of a neutral molecule [R_1H] (H_2O or CH_3COOH) involved the functional groups at C_2 in the verrucosane skeleton and successive loss of an isopropyl radical gave the ions at *m/z* 272 and 229, respectively. An ion at *m/z* 257 arised from two pathways in which a methyl radical was lost from the molecular ion and followed by loss of a neutral molecule [R_1H] involved the functional groups at C_2, or by the losses



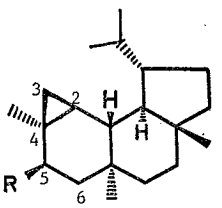
(1) R1=OH, R2=R3=H
(2) R1=OAc, R2=R3=H
(4) R1=R2=OH, R3=H
(5) R1=OH, R2=OAc, R3=H
(6) R1=R3=OH, R2=H
(7) R1=OH, R2=H, R3=OAc
(8) R1=OAc, R2=H, R3=OH
(9) R1=R3=OAc, R2=H



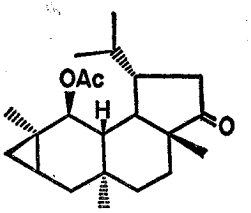
(3) R=H
(10) R=OH
(11) R=OAc



(12)



(14) R=OH
(15) R=OAc



(13)

Table I. Mass Spectral Data

Compound (**1**)
290 (7), 275 (18), 272 (38), 257 (36), 229 (24), 191 (47), 41 (base).

Compound (**2**)
336 (6), 317 (2), 290 (1), 272 (52), 257 (18), 230 (5), 229 (22), 191 (44), 177 (11), 43 (base).

Compound (**3**)
290 (18), 272 (50), 257 (17), 247 (9), 229 (24), 191 (35), 41 (base).

Compound (**4**)
306 (3), 291 (11), 288 (50), 273 (18), 270 (51), 255 (41), 245 (38), 227 (43), 189 (55), 41 (base).

Compound (**5**)
348 (2), 330 (17), 288 (6), 273 (14), 270 (68), 245 (13), 227 (31), 189 (29), 41 (base).

Compound (**6**)
306 (3), 291 (8), 288 (33), 273 (20), 270 (17), 255 (21), 245 (18), 227 (19), 220 (71), 189 (32), 176 (70), 41 (base).

Compound (**7**)
348 (4), 333 (9), 330 (19), 315 (7), 306 (7), 288 (10), 270 (51), 262 (40), 255 (30), 245 (33), 227 (17), 220 (17), 189 (54), 176 (61), 43 (base).

Compound (**8**)
348 (16), 288 (55), 270 (27), 255 (24), 245 (13), 227 (26), 189 (24), 43 (base).

Compound (**9**)
390 (4), 347 (2), 330 (16), 315 (3), 271 (22), 270 (21), 254 (17), 227 (32), 189 (28), 43 (base).

Compound (**10**)
306 (3), 288 (17), 270 (16), 255 (13), 245 (7), 227 (16), 207 (11), 189 (13), 43 (base).

Compound (**11**)
348 (5), 330 (22), 288 (23), 270 (59), 255 (34), 227 (63), 189 (46), 43 (base).

Compound (**12**)
304 (6), 289 (6), 286 (39), 271 (15), 243 (15), 218 (29), 41 (base).

Compound (**13**)
346 (39), 286 (base), 270 (32), 242 (24), 218 (26), 205 (60), 189 (54).

Compound (**14**)
290 (20), 275 (38), 272 (15), 257 (21), 248 (33), 229 (14), 191 (26), 41 (base).

Compound (**15**)
322 (19), 317 (7), 290 (6), 272 (31), 257 (21), 248 (7), 229 (14), 191 (21), 43 (base).

through the reverse order. Moreover, the 2β-mono-functional verrucosanes (**1**) and (**2**) showed commonly a characteristic ion at m/z 191($C_{14}H_{23}$), the generation of which could be rationalized by cleavages of C_1-C_2 and C_6-C_7 bonds accompanied by the transfer of a hydrogen from ring BC to A. Also, the above ion at m/z 191 courred as a base peak in low energy spectrum of 20 eV of (**1**) and suggested that the cleavages are the most facile degradation process in the verrucosane skeleton which has no substituent on ring BC.

Mass spectrum of the homoverrucosanol (**3**) (Fig. 2), which has been obtained by acid treatment of the cyclopropyl carbinol (**1**), showed a close resemblance to that of (**1**) except for a slight difference of their intensities. However, a unique fragment ion which was absent in the spectra of (**1**) and (**2**) was observed at m/z 247 with 9.5 % in relative intensity. This ion arose from a direct loss of an isopropyl radical from the molecular ion, which was proved by the observation of a metastable peak at m/z 210.4. Mass spectrum of (**3**) also exhibited the ion m/z 191 with 36 % in relatively high intensity as well as in (**1**)

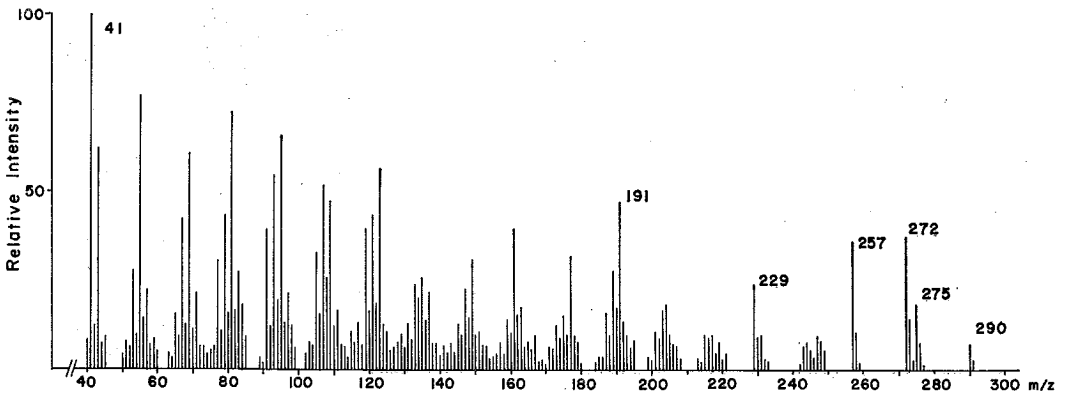


Fig. 1. Mass Spectrum of (**1**)

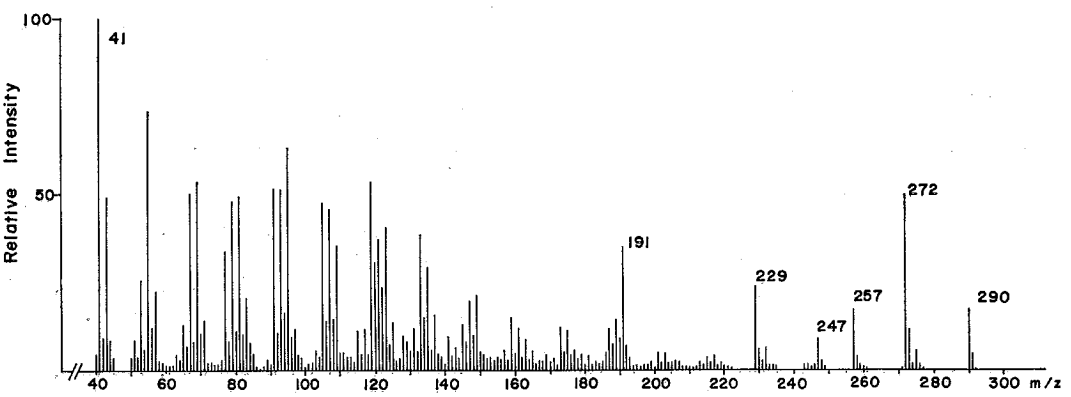


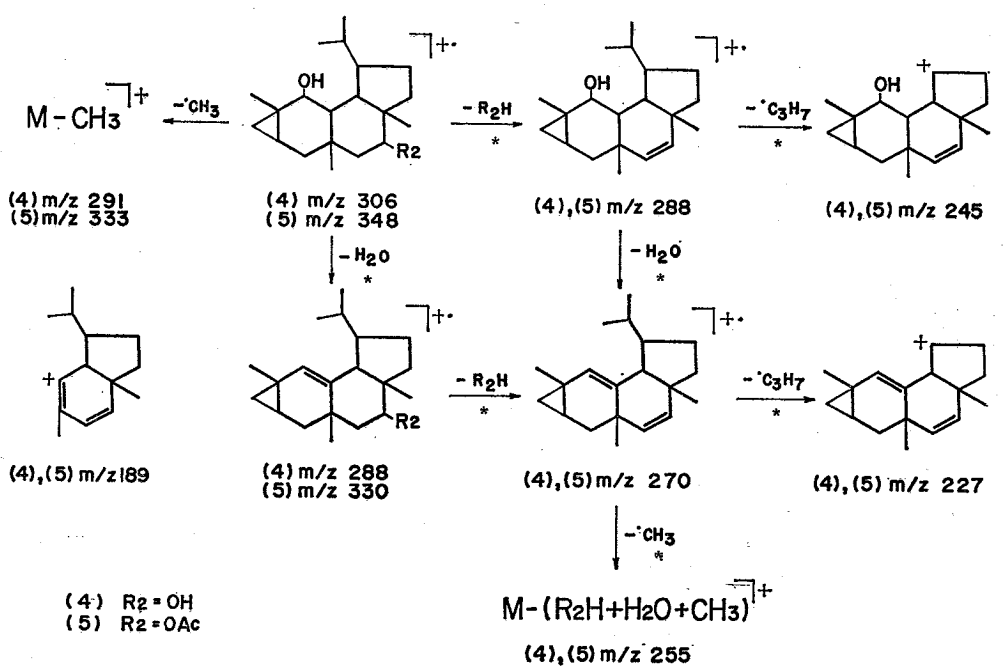
Fig. 2. Mass Spectrum of (**3**)

and (**2**).

Mass spectra of 2β, 9α-dihydoroxyverrucosane (4) and 9α-acetoxy-2β-hydroxyverruosane (5)

Mass spectra of (**4**) and (**5**) difunctionallized at C_2 and C_9 were shown, respectively, in Fig. 3 and Table I. The spectra of (**4**) and (**5**) suggested that their major fragmentation pathways are similar to those of (**1**) and (**2**). Namely, (**4**) and (**5**) lost a methyl radical or an H_2O molecule from each molecular ion to give the ions *m/z* 291 and 333 for $[M-CH_3]^+$ or *m/z* 288 and 330 for $[M-H_2O]^{+\cdot}$ as observed in (**1**). In addition, both of the spectra exhibited the ions *m/z* 288 due to loss of a neutral molecule $[R_2H]$ (H_2O or CH_3COOH) involved the functional groups at C_9 from each molecular ion and subsequent loss of an H_2O molecule gave the ion at *m/z* 270. The ion of *m/z* 270 also arose by the losses of above mentioned reverse order, successive loss of an isopropyl or a methyl radical to give at *m/z* 227 or 255. Furthermore, loss of an isopropyl radical from the fragmet ion at *m/z* 288 ($M-R_2H$) gave the ion at *m/z* 245.

In the spectra of (**4**) and (**5**), a characteristic ion due to cleavage of ring AB was observed at *m/z* 189. The ion (*m/z* 189, $C_{14}H_{21}$) may be rationalzed by fissions of C_1-C_2 and C_6-C_7 bonds accompanied by the transfer of a hydrogen from ring BC to A, after the loss of a neutral molecule $[R_2H]$ from each molecular ion. The fragmentation pathways of (**4**) and (**5**) were shown in Scheme 2.



Scheme 2.

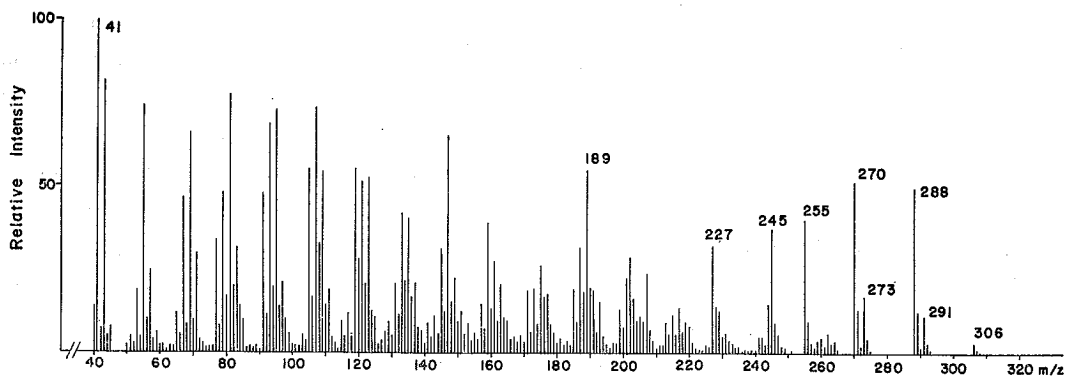


Fig. 3. Mass Spectrum of (**4**)

In the spectrum of low energy measurment at 20 eV, (**4**) also showed the fragment ion (*m/z* 189) in high intensity of 86 % exhibiting the facile cleavage of the two bonds (C_1-C_2 and C_6-C_7) in the compound having the functional groups at C_9.

Mass spectra of 2β, 11α-dihydroxyverrucosane (6), 11α-acetoxy-2β-hydroxyverrucosane (7), 2β-acetoxy-11α-hydoxy-rerrucosane (8) and 2β, 11α-diacetoxyverrucosane (9).

Mass spectra of (**6**) and (**7**) were shown in Fig. 4 and 5. Mass spectra of (**8**) and (**9**) were listed in Table I. The spectra of four compounds (**6**) ~ (**9**) difunctionallized at C_2 and C_{11} showed that their fragmentation processes are almost equal mutually. Of the spectra, major fragmentation processes of (**6**) and (**7**) were found to be the same as those of (**1**), (**2**), (**4**) and (**5**).

Spectra of (**6**) and (**7**) provided a common ion at *m/z* 270 due to the losses of the functional groups at C_{11}, which arose from the losses of an H_2O molecule at C_2 and a neutral molecule [R_3H] (H_2O or CH_3COOH) involved the functional groups at C_{11} or the losses by the reverse order. The significant ions were observed at *m/z* 176

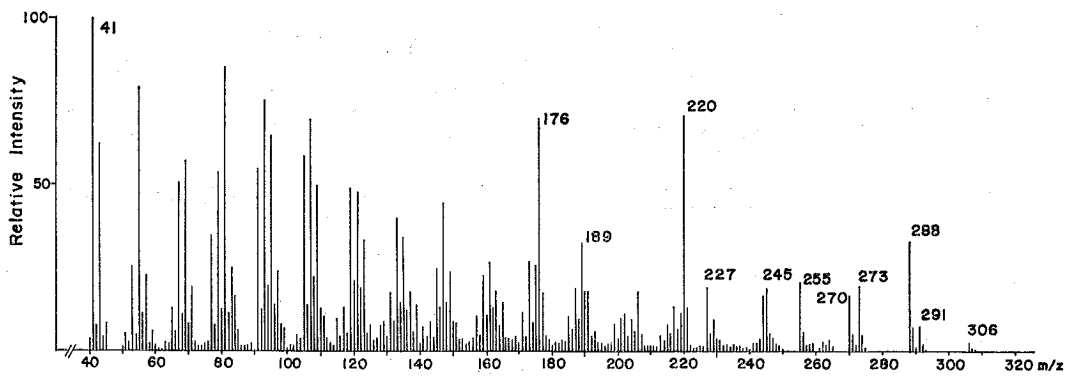


Fig. 4. Mass Spectrnm of (**6**)

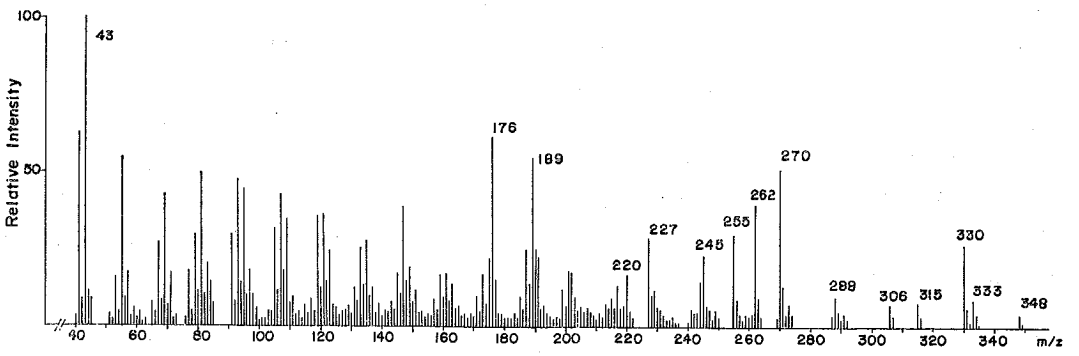


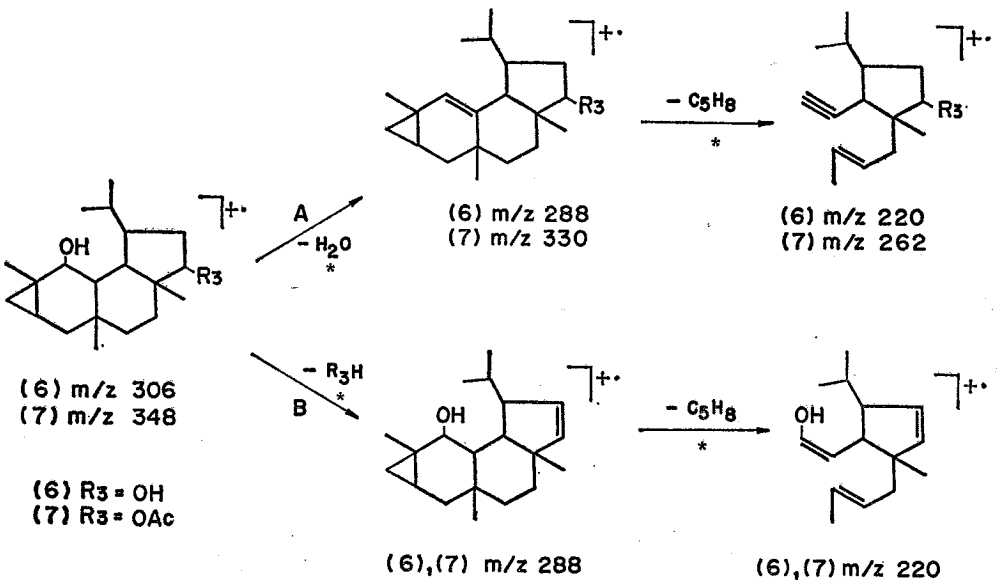
Fig. 5. Mass Spectrum of (**7**)

and 220 in (**6**) and *m/z* 176, 220 and 262 in (**7**) and the presence of these ions owed to the substituted groups at C_{11}. In (**6**), the ion at *m/z* 220 with the relative intensity of 70 % was determined by an accurate mass measurement for $C_{15}H_{24}O$, and it could be explained by an expulsion of a C_5H_8 fragment from the $[M-H_2O]$ (*m/z* 288) since a metastable peak (*m/z* 168.1 : Table II) corresponding to the above transition was detected. Another significant

Table II. Transition Assignment

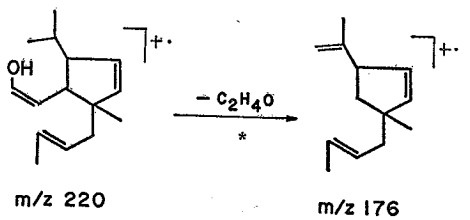
Compound	Transition	m*
(**1**)	290 ⟶ 275	260.8
	290 ⟶ 272	255.1
	275 ⟶ 257	240.2
	272 ⟶ 257	242.8
	272 ⟶ 229	192.8
(**2**)	332 ⟶ 317	302.7
	332 ⟶ 272	222.8
	317 ⟶ 257	208.4
	272 ⟶ 257	242.8
	272 ⟶ 229	192.8
(**4**)	306 ⟶ 291	276.7
	306 ⟶ 288	271.1
	288 ⟶ 270	253.1
	288 ⟶ 245	208.4
	270 ⟶ 255	240.8
	270 ⟶ 227	190.8
(**5**)	348 ⟶ 333	318.6
(**5**)	348 ⟶ 330	312.9
	348 ⟶ 288	238.3
	330 ⟶ 270	200.9
	288 ⟶ 270	253.1
	288 ⟶ 245	208.4
	270 ⟶ 255	240.8
	270 ⟶ 227	190.8
(**6**)	306 ⟶ 291	276.7
	306 ⟶ 288	271.1
	291 ⟶ 273	256.1
	288 ⟶ 273	258.8
	288 ⟶ 270	253.1
	288 ⟶ 245	208.4
	288 ⟶ 220	168.1
	270 ⟶ 255	240.8
	270 ⟶ 227	190.8
	220 ⟶ 176	140.8
(**7**)	348 ⟶ 333	318.6
	348 ⟶ 330	312.9
	348 ⟶ 288	238.3
	333 ⟶ 318	298.0
	330 ⟶ 315	300.7
	330 ⟶ 287	249.6
	288 ⟶ 273	258.8
	288 ⟶ 270	253.1
	288 ⟶ 245	208.4
	288 ⟶ 220	168.1
	270 ⟶ 255	240.8
	270 ⟶ 227	190.8
	220 ⟶ 176	140.8
(**12**)	304 ⟶ 289	274.7
	304 ⟶ 286	269.1
	289 ⟶ 271	254.1
	286 ⟶ 271	256.8
	286 ⟶ 243	206.5
	286 ⟶ 218	166.2

ion at *m/z* 176 (70 %) with elementary composition $C_{13}H_{20}$ occurred by a loss of C_2H_4O molecule from the ion at *m/z* 220, whose metastable peak was observed at *m/z* 140.8 (Table II). The above two ions at *m/z* 220 and 176 were also observed in (**7**), which is an acetate of (**6**), although the intensity (17 %) of the former ion has remarkably decreased in contrast to that of (**6**). In addition, a new ion at *m/z* 262 ($C_{17}H_{26}O_2$) corresponding to [M−86] was observed in the intensity of 40 %. From these results, possible degradation patways of the significant ions in (**6**) and (**7**) may be explained as follows (Scheme 3).



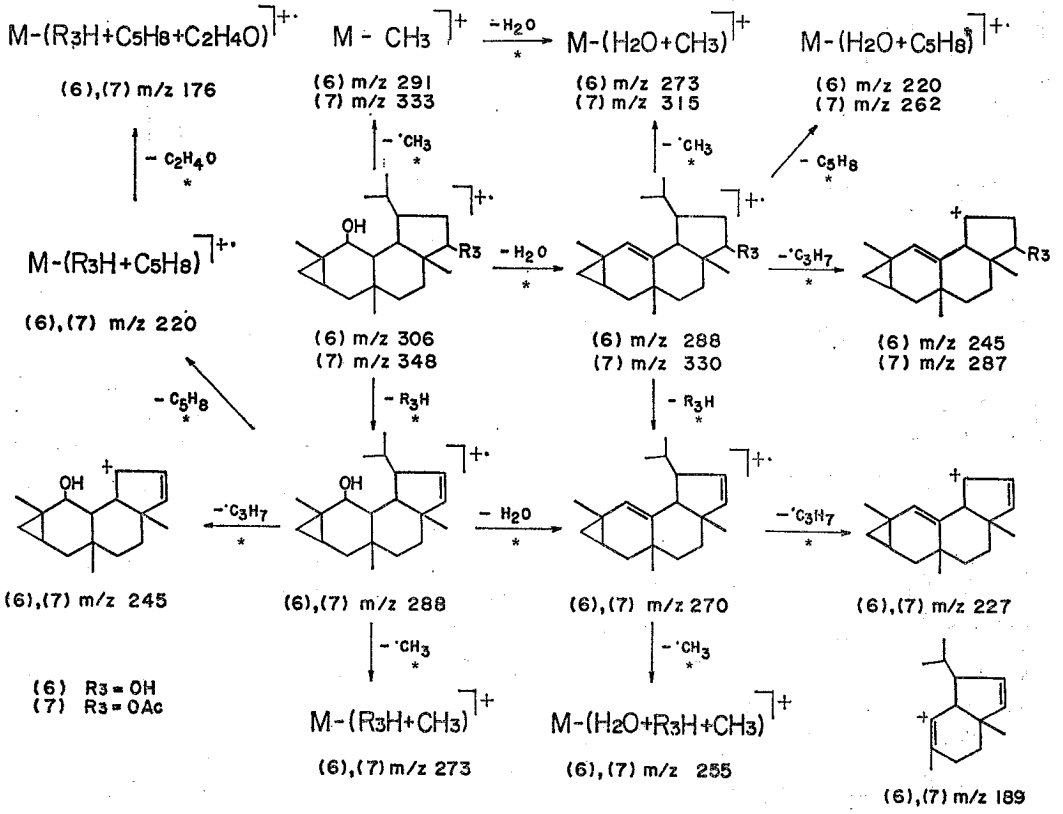
Scheme 3.

In (**6**), elimination of an H_2O molecule from the molecular ion might occur in two routes (A and B), which are loss of an H_2O molecule involving hydroxyl groups at C_2 or C_{11}. These H_2O elimination ions could then expelled a C_5H_8 fragment by the cleavages of the C_2-C_3 and C_6-C_7 bonds to give the ions at *m/z* 220 uniformely. In (**7**), route A was supported by the shifted ions at *m/z* 330 and 262, while route B showed the same mass units (*m/z* 288 and 220). The route A was thought to be a predominant reaction from the above mentioned intensities. The ion at *m/z* 176 may be formed by loss of a molecule of C_2H_4O involving C_2-hydroxyl group, C_1 and C_2 carbons from the ion of at *m/z* 220 via route B (Scheme 4).



Scheme 4.

The spectra of (**6**) and (**7**) showed the characteristic ion at *m/z* 189 as observed in (**4**) and (**5**). Hence, major fragmentation pathways of (**6**) and (**7**) were summarized in Scheme 5.



Scheme 5.

In the spectra of (**10**) and (**11**), which were prepared from (**6**) and (**7**) by treatment with acid respectively, the ion at *m/z* 189 was also observed as a characteristic ion, but no ions at *m/z* 176, 220 and 262 were observed.

In the spectrum of (**6**) in low energy impact at 20 eV, it showed the ion at *m/z* 176 as a base peak together with the ions at *m/z* 220 and 189 with 91 % and 33 %, respectively.

Mass spectrum of 2β-hydroxy-9-oxoverrucosane (12).

Mass spectrum of (**12**) was shown in Fig. 6. In the compound of (**12**) having carbonyl oxygen at C_9, the major fragmentation pathways were also similar to those of (**1**). Namely, the first pathway was successive losses of an H_2O molecule and a methyl radical from the molecular ion or the losses by the reverse order, and the second the pathway was two step losses of an H_2O mole-

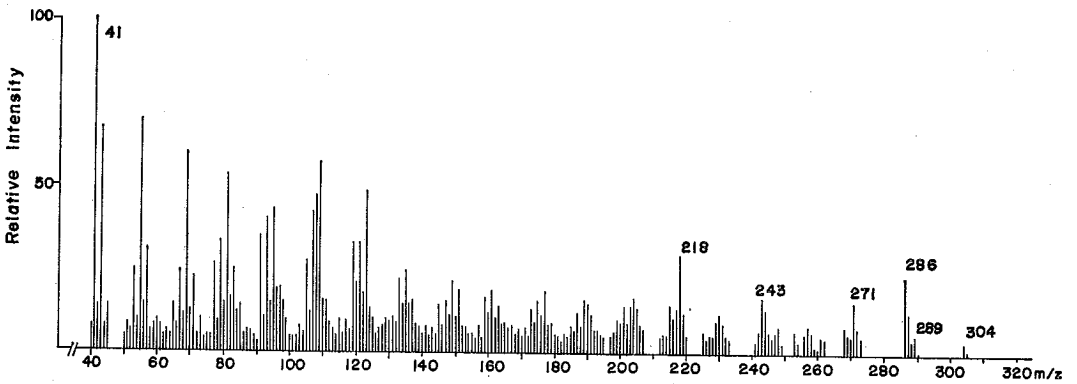
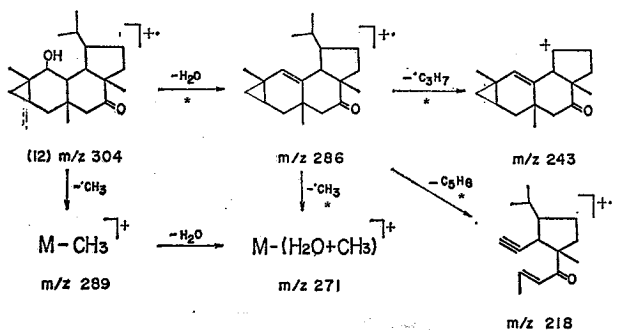


Fig. 6. Mass Spectrum of (**12**)

cule and an isopropyl radical from the molecular ion. These ions such as *m/z* 289, 286, 271 and 243 in (**14**) were shifted to 14 mass units higher due to the carbonyl group at C_9 compared with those of (**1**) (Scheme 6).



Scheme 6.

These results suggested that the carbonyl function at C_9 is stable under electron impact and the stability of the carbonyl group at C_{11} of 2β-acetoxy-11-oxoverrucosane (**13**) was similar to that of (**12**). An ion at *m/z* 218 ($C_{15}H_{22}O$, 30 %) was probably formed in two step reactions in which an H_2O molecule was expelled from the molecular ion (*m/z* 304) and subsequently C_2-C_3 and C_6-C_7 bonds were cleavaged.

Mass spectrum of 5β-hydroxyneoverrucosane (14).

Mass spectrum of (**14**), which is an isomer of (**1**) to the positions of cyclopropane ring and hydroxyl group, was shown in Fig. 7. Comparison of both spectra as shown in Fig. 1 and 7 indicated the presence of the same fragment peaks. An ion at *m/z* 248 due to a new cleavage was observed with relative intensity of 33 % and assigned to the ion of $[M-C_3H_6]^{+\cdot}$. In the spectrum of (**15**) which is an acetyl derivative of (**14**), an ion at *m/z* 248 was also obseved and this ion corresponded to $[M-CH_2CO-C_3H_6]^{+\cdot}$. These facts provided evidence for a direct loss of a C_3H_6 molecule from molecular ion in (**14**), and the direct loss was confirmed by the observation of the metastable peak at *m/z* 212.1 (*m/z* 290 → 248). The above ion at *m/z* 248 was a characteristic ion in (**14**), the genesis of *m/z* 248 may be a result from elimination of the C_3H_6 molecule involving a hydrogen transfer from the isopropyl function at C_{13}.

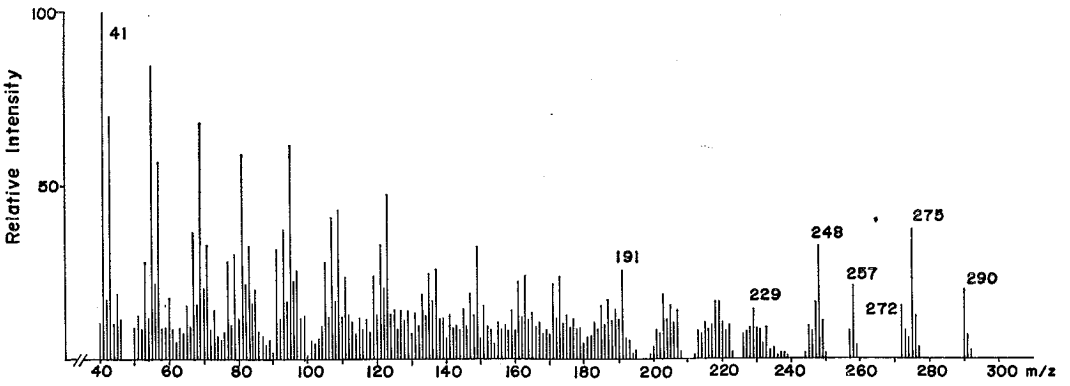


Fig. 7. Mass Spectrum of (**14**)

It was found that formation of the ion at *m/z* 248 is facile because of the observation of 69% even in the spectrum of 20 eV of (**14**).

3. Conclusions

In the spectra of all verrucosane derivatives, characteristic ions which correspond to the fission of ring A and located charge in the ring BC side were observed. Spectra of the compounds having two functional groups at C_2 and C_9 or C_{11} were clearly different from the spectra of the monofunctionallized compounds at C_2. Mass spectral investigation of the diterpenoids of the verrucosane, neoverrucosane and homoverrucosane types provided a useful way for the structral determination of these diterpenoids having the difference with respect to the kind and the position of functional group.

4. Experimental

The high resolution mass spectra were taken using a Hitachi RMU-7L double focussing mass spectrometer; ionizing energy 70 eV, ionizing current 80 μA, and accelerating voltage 3.2 kV. Relative intensities of the fragment ions were calculated based on the ordinary mass spectra taken by a Hitachi RMU-6 mass spectrometer. The samples were obtained according to the original papers.[1-5] They were purified by means of column chromatography and confirmed by nuclear magnetic reso nance spectra.

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Keywords
EIMS
Fragmentation pathway
Metastable ion
Fifteen diterpenoids