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HIGH RESOLUTION MASS SPECTRA OF GIBBERELLINS

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Up to now, sixteen gibberellins have been isolated and their structure established. Mass spectroscopy of these large number of gibberellin homologues should afford useful information not only for identification but also for structural elucidation of new gibberellins.

Recently Wulfson et al. (1) reported the mass spectra of methyl esters of gibberellins A_1, A_3 and A_4, and discussed possible fragmentation process.

Now we wish to present our result concerning high resolution mass spectra of twelve C_{19} gibberellin homologue methyl esters shown in Fig. 1. Our objective in this research is to determine fragmentations, which are characteristic to the common structural features, as well as structural modifications in gibberellins.

The mass spectra were obtained by Mattauch-Herzog type spectrographs (CEC 21-110B and JNS-01S), with direct inlet system, under following conditions: Electron accelerating voltage, 70 eV; temperature of ionization source, 140-160°; temperature of vaporization, 100-130°. Based on Biemann's procedure (2), element maps were prepared.

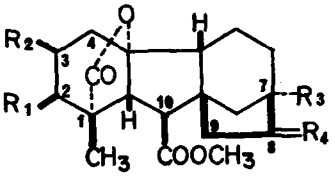
Results are summarized in Table 1, in which compositions of prominent fragment ions in high mass region are expressed as M-X (X:lost mass). Most C_{19} gibberellin methyl esters exhibited parent peaks having moderate intensity, and prominent peaks due to M-32(CH_4O), M-46(CH_2O_2), M-60($C_2H_4O_2$), M-78($C_2H_6O_3$),

TABLE 1.

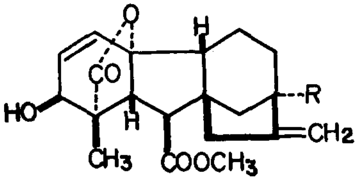
		A_1-Me	Dihyd. A_1-Me	A_2-Me	A_3-Me	A_4-Me	A_5-Me	III	II	A_7-Me	A_8-Me	A_9-Me	I
0	(M^+)	****	*****	**	****	**	****	****	****	**	******	*****	**
17	HO	*	**	*	*	-	-	-	-	**	*	-	-
18	H_2O	**	****	***	***	*	-	*	*	****	**	-	-
28	CO	-	-	-	-	-	*	**	-	*	*	-	-
32	CH_4O (CH_3OH)	*****	*****	*****	****	****	****	***	***	******	******	******	****
41	C_3H_5	-	***	*	-	-	***	*	*	-	-	-	-
43	C_3H_7	-	******	-	-	-	-	*****	*****	-	-	*	**
44	CO_2	-	-	-	*	-	*****	*****	*	**	-	*****	*
46	CH_2O_2 (HCOOH)	***	****	**	***	*	*	*	***	****	***	****	**
50	CH_6O_2 (CH_3OH, H_2O)	**	***	****	*	*	-	*	-	***	****	-	-
60	$C_2H_4O_2$ ($HCOOCH_3$)	****	****	*****	**	**	**	***	***	*****	*****	******	***
62	CH_2O_3 (H_2O, CO_2)	**	****	**	***	*****	***	***	-	*****	*	-	-
64	CH_4O_3 (H_2O, HCOOH)	***	***	***	*	*	-	-	-	**	***	-	-
76	$C_2H_4O_3$ (CH_3OH, CO_2)	**	**	***	*	*	***	***	**	***	**	-	*
78	$C_2H_6O_3$ (CH_3OH, HCOOH or $HCOOCH_3$, H_2O)	****	****	****	***	**	**	***	***	***	****	*	*
104	$C_3H_4O_4$ ($HCOOCH_3$, CO_2)	**	***	***	**	*	******	******	****	****	**	*****	***
106	$C_3H_6O_4$ ($HCOOCH_3$, HCOOH)	***	***	***	***	*	**	**	**	*****	***	****	****
122	$C_3H_6O_5$ ($HCOOCH_3$, H_2O CO_2)	****	****	****	*****	******	**	***	*	******	****	/	*
124	$C_3H_8O_5$ ($HCOOCH_3$, H_2O HCOOH)	***	**	**	***	*	-	-	-	**	***	/	*
139	$C_3H_7O_6$ ($HCOOCH_3$, H_2O CO_2, OH)	**	**	****	****	/	/	/	/	/	***	/	/

Relative intensity (logarithmic scale) is represented in numbers of asterisk (base peak above m/e 130 in each gibberellin methyl ester : six asterisks).

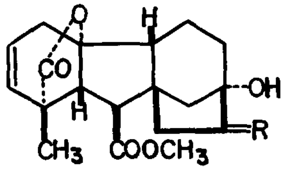
M-104($C_3H_4O_4$), M-106($C_3H_6O_4$) and M-122($C_3H_6O_5$) fragment ions. These ions constitute a characteristic pattern which can be associated with the common structural features of gibberellins. Of these, M-32 and M-60 may be formed by elimination of CH_3OH and $HCOOCH_3$ from C-10 methoxycarbonyl group, as suggested by Wulfson et al. M-46 and M-62 ions may be due to elimination of HCOOH and CO_2+H_2O from C-1 lactone. Other peaks can be explained in terms of participation of both C-1 lactone and C-10 methoxycarbonyl groups.



A_1-Me	$R_1=R_3=OH$, $R_2=H$, $R_4=CH_2$
dihydro A_1-Me	$R_1=R_3=OH$, $R_2=H$, $R_4=H$, CH_3
A_2-Me	$R_1=OH$, $R_2=R_3=H$, $R_4=OH$, CH_3
A_4-Me	$R_1=OH$, $R_2=R_3=H$, $R_4=CH_2$
A_8-Me	$R_1=R_2=R_3=OH$, $R_4=CH_2$
A_9-Me	$R_1=R_2=R_3=H$, $R_4=CH_2$
I (3)	$R_1=R_2=H$, $R_3=OH$, $R_4=CH_2$
II	$R_1=R_2=H$, $R_3=OH$, $R_4=H$, CH_3



A_3-Me	R=OH
A_7-Me	R=H



A_5-Me	$R=CH_2$
III	R=H, CH_3

FIG. 1.

Hydrocarbon ion peaks with the largest number of carbon atoms are $C_{17}H_{16-24}$ fragment ion groups. They can be formed from elimination of hydroxyl, lactone and methoxycarbonyl groups from original molecules. These fragment ions can be correlated to the basic skeleton of gibberellin groups, furnishing evidence to the presence of gibbane skeleton. Fragments, $C_{17}H_{15-25}O$, also exist in various intensity. In A_1-, dihydro A_1-, A_3-, A_5-, A_8-Me, I, II and III, which contain a hydroxyl group at C-7, the intensity of $C_{17}H_{15-25}O$ ions is stronger than that of $C_{17}H_{16-24}$, while in A_2-, A_4-, A_7- and A_9-Me containing no C-7 hydroxyl group, their intensity is reversed as shown in Table 2. Thus, the comparison of intensity between $C_{17}H_{16-24}O$ and $C_{17}H_{15-25}$

TABLE 2.

Methyl Esters of Gibberellin containing C-7 Hydroxyl Group

	A_1-Me	Dihydro A_1-Me	A_3-Me	A_5-Me	III	II	A_8-Me	I
$C_{17}H_{17-23}$	17**		17*	17**	17*		17***	17*
	18*		18*	18**			18*	18*
	19**	19**		19***	19****	19*		19*
	20*	20***		20*	20***			20*
		21**				21**		21*
					22*	22*		
						23*		
$C_{17}H_{15-25}O$			15*					
	16*		16***					
	17***		17*****	17*	17*		17****	
	18***		18*****	18*			18****	
	19****	19**	19****	19***	19*		19***	19*
	20****	20**	20**	20*****	20**		20**	20****
	21****	21****		21****	21*****	21**	21*	21****
	22*	22****		22*	22******	22**		22***
		23****		23*	23***	23****		23***
					24***	24****		
						25***		

Methyl Esters of Gibberellin lacking C-7 Hydroxyl Group

	A_2-Me	A_4-Me	A_7-Me	A_9-Me
$C_{17}H_{16-24}$			16**	
	17*		17*****	
	18**	18*	18******	18*
	19***	19***	19******	19***
	20****	20******	20*****	20****
	21****	21*****	21****	21*****
	22***	22***	22**	22*****
				23******
				24*****
$C_{17}H_{17-23}O$			17***	
			18*****	
	19*	19*	19*****	19*
	20**	20*	20***	
	21***	21**	21****	
	22***	22***	22***	22****
	23***			

ion peaks makes the determination of presence of the hydroxyl group at C-7 possible.

Some gibberellins show M-44(CO_2) peak, occurrence of which can be ascribed to participation of C-1 lactone as M-46 peak. From a comparison of intensity between M-44 and M-46 peaks, it is now evident that A_5-Me and III, which contain C-2~C-3 double bond show intense M-44 but very weak M-46 peaks, while A_1-, dihydro A_1-, A_2-, A_4- and A_8-Me, which contain C-2 hydroxyl, exhibit much stronger M-46 but no M-44 peaks. In A_9-Me, I and II, which lack C-2 hydroxyl, as well as A_3- and A_7-Me, which contain C-2 hydroxyl and C-3~C-4 double bond, both M-44 and M-46 peaks are observed in appreciabe intensity. This result may suggest that C-2~C-3 and C-3~C-4 double bonds facilitate elimination of CO_2 but C-2 hydroxyl group induces elimination of HCOOH from C-1 lactone. This observation offers useful information about the presence of the hydroxyl group at C-2.

A_5-Me and III show M-41(C_3H_5) peak, which can be ascribed to loss of C-1, C-3 and C-4 from ring A. Dihydro A_1-Me, II and III also show intense peak with composition of M-43(C_3H_7). This may be due to loss of C-8, C-9 and C-8 methyl from ring D, because only the derivatives whose C-8 exocyclic methylenes have been reduced show this intense peak.

Position of hydroxyl groups in gibberellin groups are limited at C-2 and C-7, and rarely at C-3 and C-8. Gibberellin methyl esters containing hydroxyl at C-2 give rise to M-18 ions with moderate intensity, while those containing hydroxyl only at C-7 show no peak or rather weak peak of M-18, intensity of which is sensitive to conditions for measurement.

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2. K. Biemann, P.H.U. Bommer and D. Desiderio, Tetrahedron Letters, **1964**, 1725.
3. I was isolated from immature seeds of *Pharbitis nil* and its structure was established on the basis of chemical and physico-chemical evidences (to be published).