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MASS SPECTROMETRY OF GIBBERELLINS

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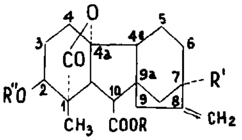
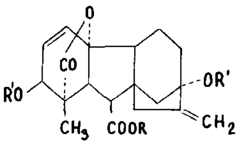
The method of mass spectrometry had been successfully applied to a great number of natural compounds incl.diterpenes /1/, but so far it had not been extended on gibberellins. We investigated the pattern of fragmentation upon electron impact for some representatives of this group, namely, for derivatives of gibberellin A_1 (I-IV), gibberellin A_3 (V-VII) and gibberellin A_4 (VIII). It was hoped to take advantage of this method for the analysis of mixtures of gibberellins (e.g. gibberellins A_1 and A_3) which are difficult to separate chromatographically.



(I) $R=CH_3$, $R'=OH$, $R''=H$
(II) $R=C_2H_5$, $R'=OH$, $R''=H$
(III) $R=CH_3$, $R'=OD$, $R''=D$
(IV) 3,4-d_2, $R=CH_3$, $R'=OH$, $R''=H$
(VIII) $R=CH_3$, $R'=H=R''$



(V) $R=CH_3$, $R'=H$
(VI) $R=C_2H_5$, $R'=H$
(VII) $R=CH_3$, $R'=D$

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The mass spectra of derivatives of gibberellin A_1 (I-IV) and gibberellin A_4 (VIII) exhibit a noticeable peak of molecular ion (see fig.Ia and 2). There are also (see scheme I) $M-H_2O$ (ions a_1-a_3) and M-ROH peaks (ions b_1 and b_2), the latter of these being of high intensity. In case of gibberellin A_3 derivatives (V-VII) the M-ROH peak is relatively small (see fig.Ib).

The absence of alkoxy group in ions b_1 and b_2 follows from the examination of the mass spectra of esters (I) and (II). Whilst in the latter case the molecular ion peak is shifted from m/e 362 to m/e 376, the mass number of ion b_1 (m/e 330) remains unaffected.

The degradation of molecular ions from (I) and (VIII) proceeding via elimination of $HCOOCH_3$ leads to ions c_1 and c_2, correspondingly (see scheme I). The structure of ion c_1 is confirmed by the mass spectra of di-O-deuteroderivative (III) and ethyl ester (II). In the first case the mass number of ion c_1 increases by two mass units, whilst in the spectrum of (II) there is no change in the position of this peak. Successive elimination of H_2O and CO_2 from ions c_1 or c_2 gives rise to ions d_1 or d_2 and e_1 or e_2 correspondingly. The structures of ions d_1 and e_1 are corroborated by the mass spectra of (II), (III) and 3,4-d_2-derivative (IV). Ions d_1 and d_2 may also originate from ions a_1 and a_3 upon the loss of HCOOR.

The mass spectrum of ester (I) contains an abundant peak at m/e 275(ion f_1) which is not present in the spectra of (V) and (VIII). The structure of this ion requires further elucidation. However, it may be considered as certain that fragment f_1 contains C-2, C-7 and C-10 atoms with corresponding groups, since in the spectra of (II) and (III) its mass number is shifted 14 or 2 mass units upwards correspondingly.

The comparison of the mass spectra of (I) and (V) shows that in the latter case the mass numbers of fragments a_1, b_1, c_1, d_1 and e_1 decrease by two mass units, which is in line with the difference in molecular weights of these two compounds.

It implies the similarity of the main fragmentation patterns for derivatives of gibberellin A_1 (e.g.I) and A_3 (e.g.V).

Unlike the mass spectra of (I), (II) and (VIII), in the spectra of gibberellin A_3 derivatives (V and VI) the base peak appears at m/e 136 (ion g, see scheme 2). This fragment results probably from the cleavage of the allylic 4a-4b bond combined with the fission of the bond 9a-10. The fact that ion g contains rings C andD as well as 7-OH group is confirmed by the mass spectra of (VII) and (VIII). In the spectrum of (VII) its mass number is shifted to m/e 137 whilst in that of (VIII) the m/e 136 peak is absent.

These preliminary results show the applicability of mass spectrometry to the identification of gibberellins A_1, A_3 and A_4 both in individual state and in mixtures (in form of their esters).

All mass spectra were obtained on the commercial MX-1303 mass spectrometer. The instrument was furnished with an all-glass inlet system allowing the sample to be charged directly into the ion source, near the ionisation chamber, the ionisation energy being 30 eV and temperature range 115-160° (±1°, temperature stabilization).

Esters (I), (II), (V) and (VI) were prepared from pure samples of gibberellins A_1 and A_3 on treatment with corresponding diazoalkanes. Gibberellin A_1 had m.p.253-255° and $\alpha_{D}^{20}+36^{\circ}$ /cf.2/; gibberellin A_3 had m.p.233-235° and $\alpha_{D}^{20}+86^{\circ}$ /cf.3/. Di-o-deutero-derivatives (III) and (VII) were obtained from (I) or (V) on repeated boiling with CH_3OD. (3x3 hrs.). Methyl ester of 3,4-d_2-gibberellin A_1 (IV) was obtained according to /2/, the only difference being the use of gaseous D_2 instead of H_2. The compounds thus prepared had following m.p.p: (I) 231-233°; (V) 208-210°; (II) 161-165°; (VI) 153-155°; (III) 224-230°; (VII) 206-208°. Ester (IV) had m.p.229-234° (from hexane-ethyl acetate) and $\alpha_{D}^{20}+43^{\circ}$ (c 0.65 in C_2H_5OH). The sample of gibberellin A_4 (VIII) obtained by method /3/, was kindly provided by Dr.I.A.Gurvich, to whom we express our thanks.

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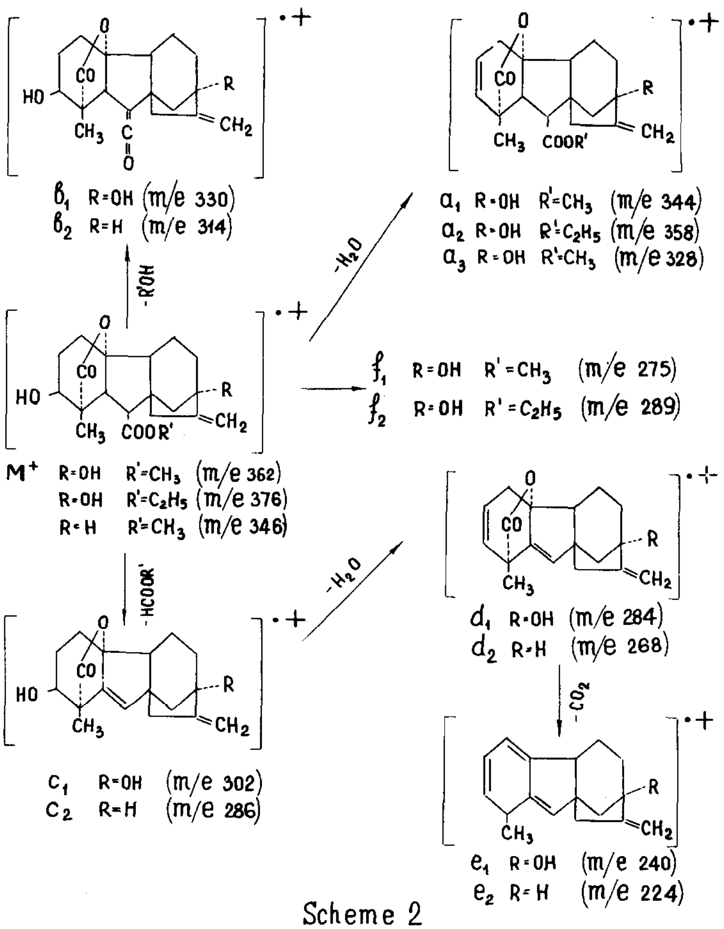
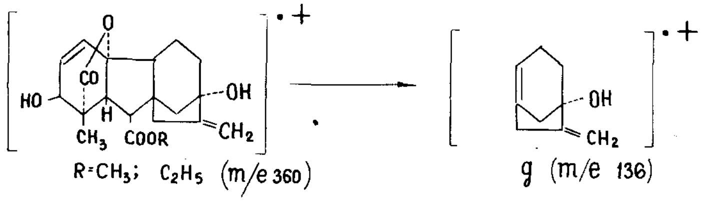
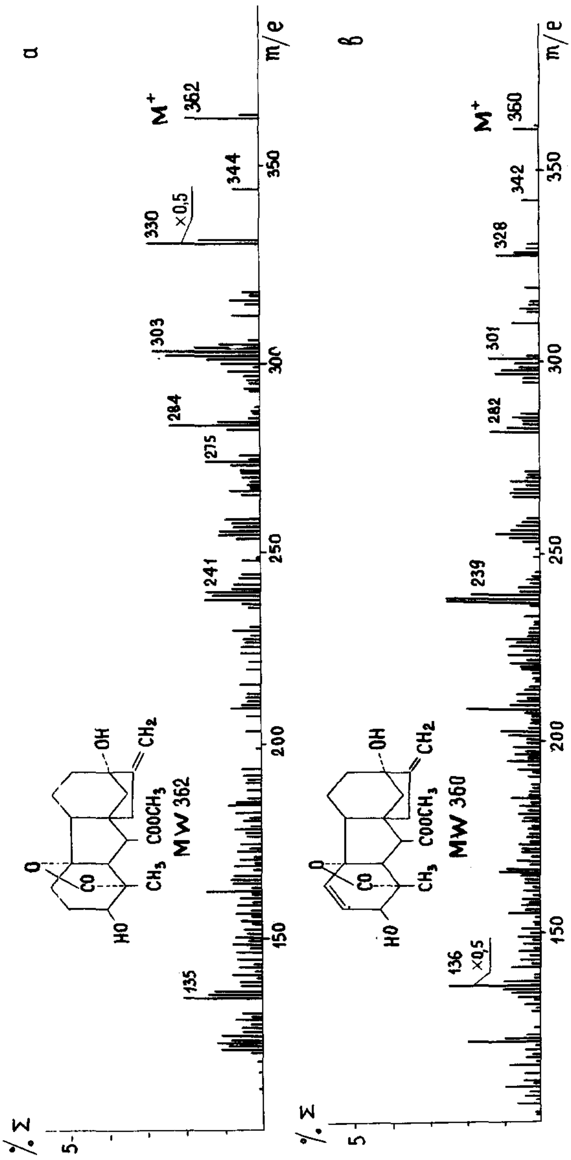
Scheme 1



Scheme 2




a
β
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m/e
M+
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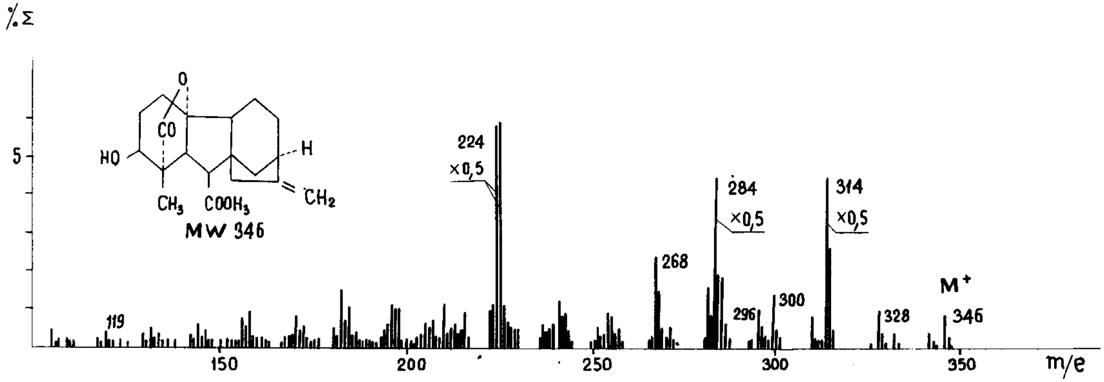
344
330
×0,5
303
284
275
241
135
MW 362
360
342
328
301
282
239
136
MW 360
150
200
250
300
350
HO
OH
CH2
CH3
COOCH3
CO
O

Fig. 1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CH₂
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284
×0,5
314
×0,5
268
296
300
328
M⁺
346
150
200
250
300
350
m/e

Fig. 2

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