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174. Tsutomu Furuya,[*1] Hisashi Kojima,[*1] and Hiroshi Sato[*2] : The Analysis of Furanocoumarins by the Combination Instrument Gas Chromatograph and Mass Spectrometer.[*3]

(*College of Pharmaceutical Sciences, Kitasato University,*[*1]
and The Naka Works of Hitachi, Ltd.[*2])

The application to furanocoumarins analysis by the homemade combination instrument gas chromatograph and mass spectrometer was described and the ease of the identification of natural products from these mass spectra was demostrated.

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During the last few years increasing attention has been drawn to the direct introduction[1~9)] of the effluent from a gas chromatograph column into a mass spectrometer.

The combination instruments[10,11)] of a high resolution rapid scanning mass spectrometer and a capillary column gas chromatograph were recently developed by Hitachi's group in our country, and have been used to assist in the identification and structure elucidation of natural products.

This paper describes with the application of the combination instrument to the analysis of furanocoumarins from the root of *Heracleum lanatum* Michaux var. *nipponicum* Hara (Umbelliferae) and the discussion on the fragmentations of three furanocoumarins.

Experimental

Instrument and Operating Conditions——Hitachi RMU-6D mass spectrometer was connected to Golay column in Hitachi F-6D gas chromatograph through Watson-Biemann type sample enrichment device.[3,6)] Continuous registration of the total ion current serves as the gas chromatographic record.

Golay column (45 m. long, 0.5 mm. inner diameter) was coated with Apiezon L. The helium pressure was 1.5 kg./cm^2, giving a flow rate of about 0.5 ml. per minute. Injection block was 300° and gas chromatography was run programmed linearly at the rate of 10° per minute from 180° to 210°. The total emission current of mass spectrometer was 80 μ amp, ion accelerating voltage 1800 volts, electron accelerating voltage 70 volts, and ion source temperature 250°.

Sample size used was 1 μl. of the acetone solution of neutral fraction which was prepared from the dried root (30 gram) of *Heracleum lanatum* Michaux var. *nipponicum* Hara according to Svendsen's method.[12)]

[*1] Shiba, Minato-ku, Tokyo (古谷 力, 児嶋 脩).

[*2] Katsuta, Ibaraki-ken (佐藤 弘).

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Results and Discussion

The fine gas chromatogram of furanocoumarins from the root of *Heracleum lanatum* Michaux var. *nipponicum* Hara is shown in Fig. 1. The separation of sphondin from bergapten was not successful, but the good separation has been obtained by authors[13] using 12% HI-EFF-1BP on Gas Chrom P (80~100 mesh)-packed column.

Mass spectra as shown in Fig. 2, 3 and 4 were nicely recorded when each peaks were attained on their tops.

Recently mass spectrometry has extensively been applied to the study of coumarins,[14~17] and furanocoumarins[14] such as angelicin,[18] xanthotoxin,[18] bergapten,[18,19] iso-

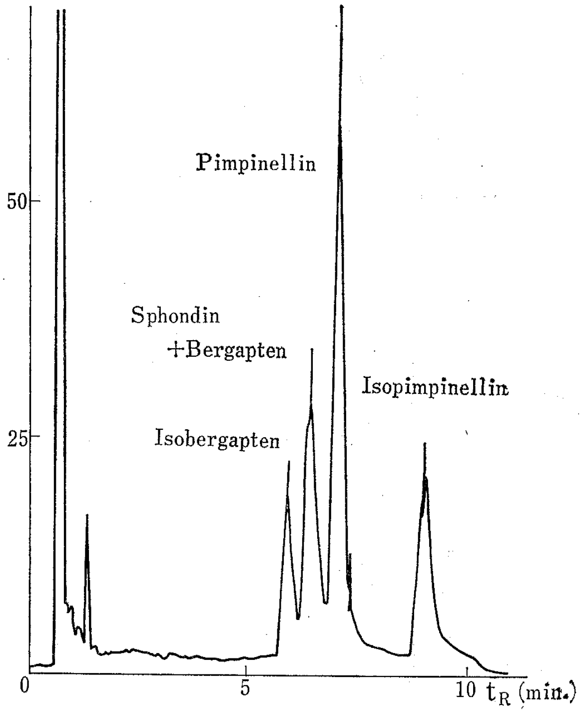

Fig. 1. Gas Chromatogram of Furanocoumarins from the Root of *Heracleum lanatum* Michaux var. *nipponicum* Hara

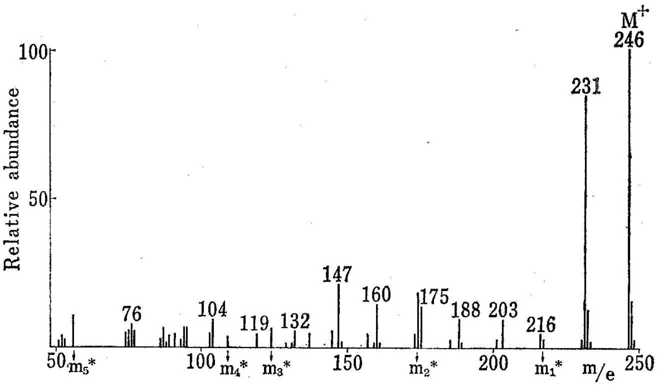

Fig. 3. Mass Spectrum of Pimpinellin

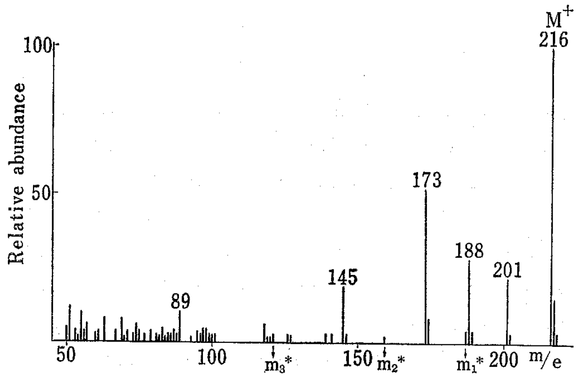

Fig. 2. Mass Spectrum of Isobergapten

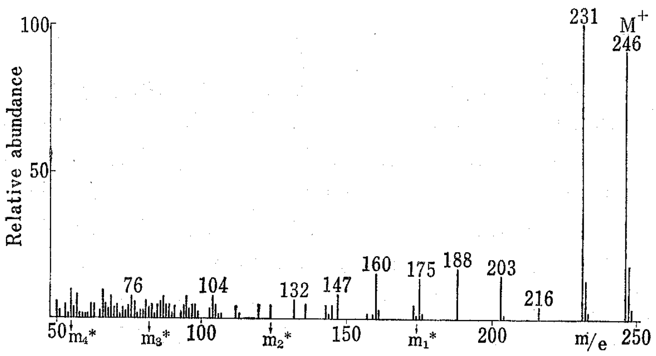

Fig. 4. Mass Spectrum of Isopimpinellin

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pimpinellin,[18,19] imperatorin,[19] isoimperatorin,[19] oxy-peucedanin,[19] prangenin[19] and archangelin.[20]

We have also studied the possible fragmentations of mass spectra of isobergapten and pimpinellin, which have not yet been determined, and moreover, the full fragmentation of isopimpinellin, which has partially determined.[18,19]

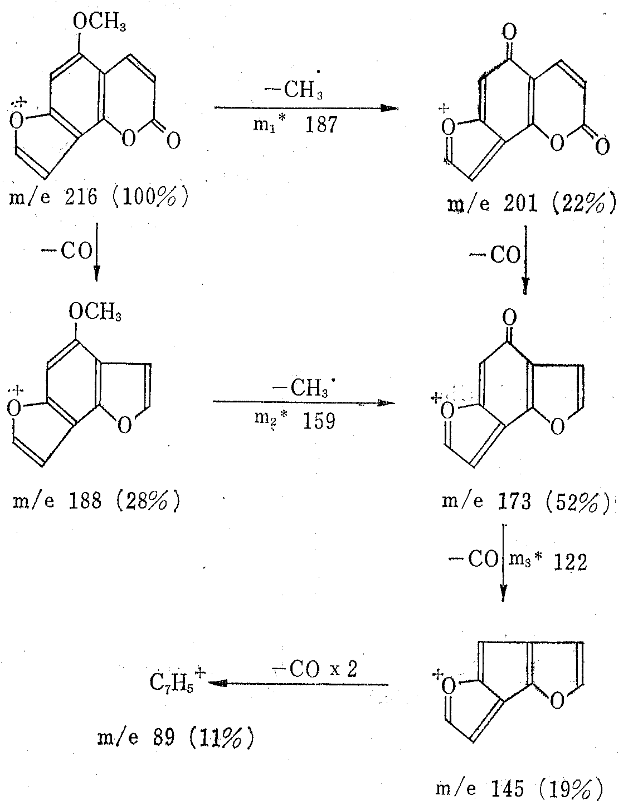

Chart 1. Proposed Fragmentation of Isobergapten

The localizations of charge in structures are not clear.

Isobergapten molecular ion (base peak) displayed the ready elimination of one molecule of carbon monoxide to give benzofuran ion at m/e 188 (28%), and also the loss of methyl radical from aromatic methoxyl group as shown by the metastable transition $[216]^+ \rightarrow [201]^+ + 15 [m_1^* =$ m/e 187 (Calcd. 187.0)] gave rise to conjugated oxonium ion at m/e 201 (22%). From the both ions described above, the elimination of methyl radical and carbon monoxide respectively gave quinonoid ion at m/e 173 (52%), which followed by the removal of carbon monoxide to give rise to cyclopentene ion at m/e 145 (19%). Plausible formulations for the most intensive peaks are shown in Chart 1.

The fragmentation of pimpinellin is given in Chart 2.

Pimpinellin gave strong molecular ion as base peak. The ready loss of methyl radical from aromatic methoxyl group as shown by the metastable peak at $m_1^* =$ m/e 217 (Calcd. 216.9) due to the transition $[246]^+ \rightarrow [231]^+ + 15$ gave *p*-quinonoid ion, which would be expected to be preferred energetically over ion in parenthesis.[21]

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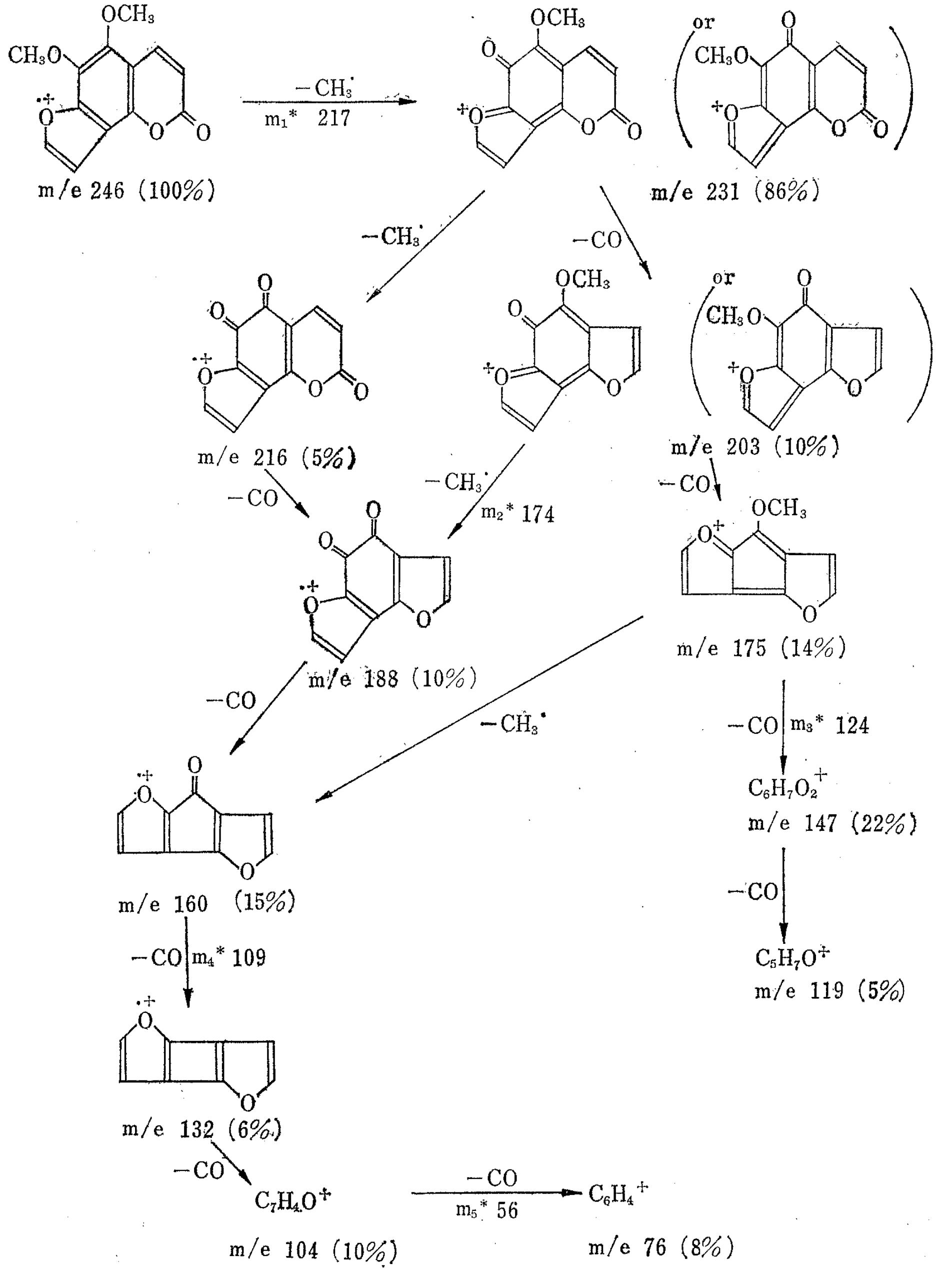

Chart 2. Proposed Fragmentation of Pimpinellin

Furthermore, the elimination of methyl radical from methoxyl group and carbon monoxide from lactone ring occurred alternately to give *o*-quinonoid ion at m/e 188 (10%), which followed by the successive loss of carbon monoxide to derive ion at m/e 160 (15%) and ion at m/e 132 (6%). On the other hand, ion at m/e 160 also would be probably originated from ion at m/e 203 (10%) *via* ion at m/e 175 (14%). The metastable ions such as m_2^*=m/e 174 (Calcd. 174.1), m_3^*=m/e 124 (Calcd. 123.5), m_4^*=m/e 109 (Calcd. 108.9), and m_5^*=m/e 56 (Calcd. 55.5) were observed clearly.

Isopimpinellin showed likewise mass spectrum as shown in Chart 3.

The relative abundance of parent ion was 91% and (M–15) ion at m/e 231 gave base peak, which was different from that of pimpinellin. We also were able to observe other many fragment ion peaks due to the removal of methyl radical from methoxyl group followed by the successive loss of carbon monoxide.

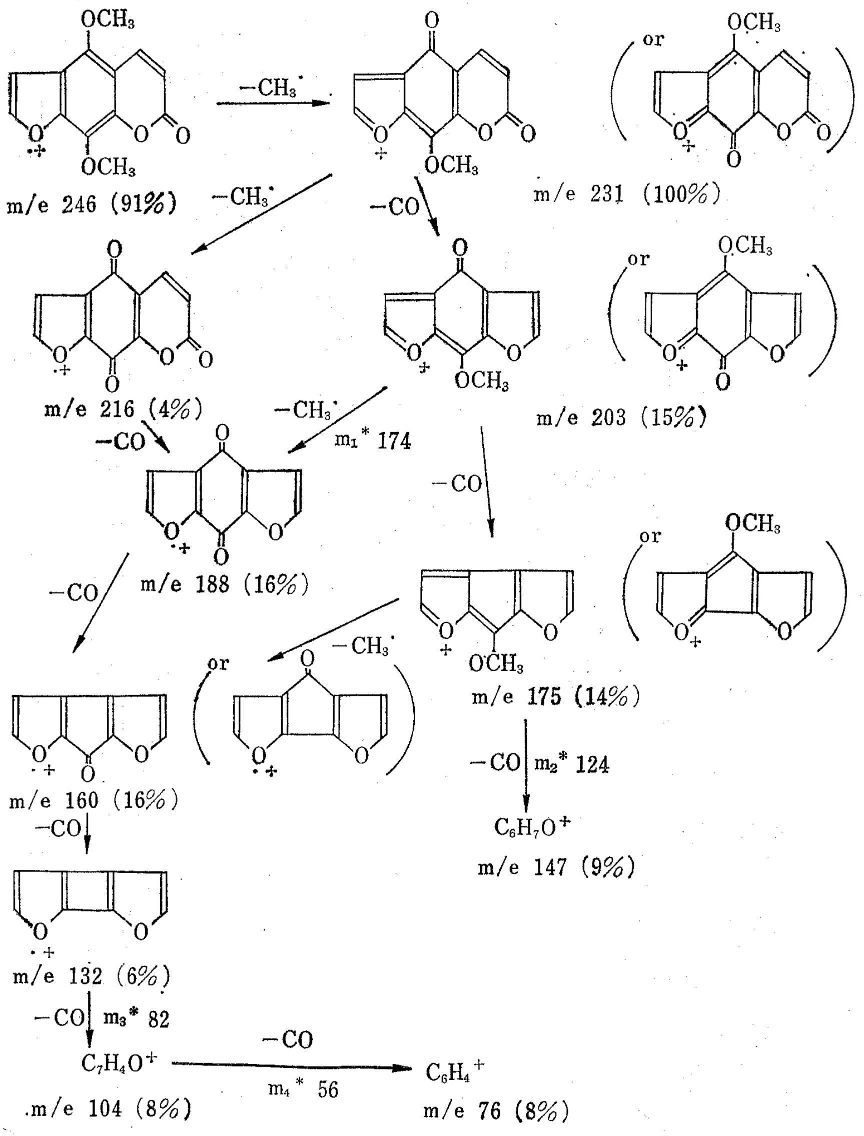

Chart 3. Proposed Fragmentation of Isopimpinellin

The characteristic fragmentations of furanocoumarins under electron impact were the ready loss of carbon monoxide from the lactone ring to form ion having the benzofuran structure followed by the further loss of the remaining oxygen atom, again as carbon monoxide, and the ready elision of methyl radical from aromatic methoxyl group to give rise to conjugated oxonium ion.