Note

An Application of the Fitness Index Method; Identification of Skatole from the Bethyloid Wasp *Cephalonomia gallicola* by Mass-spectrometry

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In previous paper,¹⁾ the average fitness index was developed as a mass-data comparison method, enabling the methylated sex pheromone of the varied carpet beetle *Anthrenus versicolor*²⁾ to be reconfirmed as methyl 5-undecenoate, by comparing the indices of all positional isomers of methyl undecenoate against the methylated pheromone. In order to confirm the feasibility of the method, the methyl position of the natural methylindole was determined experimentally, because the natural skatole (3-Me*), which is known as a component of mandibular gland secretion of the bethyloid wasp *Cephalonomia gallicola*,³⁾ was currently available as well as all the isomers of methylindoles. It was practically impossible to identify the methylindoles only by simple comparison of the EI-mass spectra among their isomers, because those indoles, except for 1-Me, gave almost the same EI-mass spectra. Gas liquid chromatography (GC) with a conventional packed column could not separate each isomer of methylindole, except for 1-Me. The only method hitherto applicable to identify each methylindole without isolation is by using GC with an appropriate capillary column. All the isomers of methylindole can be separated by GC on a Chromopack capillary column (CP Wax 57CB, 0.21 mm i.d. $\times$ 25 m, at 180°C and a split ratio of 50:1).³⁾ Therefore, the application of the fitness index method to methylindoles seems to be valuable as an alternative identification method.

Seven isomers of methylindoles were dissolved in ether as 100 ppm solutions for GC/EI-MS measurement. The natural methylindole was extracted from bethyloid wasps (ca. 40 ml by volume) by pentane and used for the analysis

at an appropriate concentration after partial purification using a silicic acid column.³⁾ The mass spectra of all compounds were independently measured five times at the same conditions and by the reported methods³⁾ using a Hitachi M-80B GC/MS spectrometer at 70 eV on a glass column (3 mm i.d. $\times$ 1 m) packed with Chempack FP (Gasukuro Kogyo Inc.) at 170~190°C and with 30 ml/min helium flow rate. The retention times of all the methylindoles were maintained to around 1 min by adjusting the oven temperature. The sample size for each measurement was also adjusted to give the base ion intensity counts in a range between 524,711 and 189,167. The intensity data (%) of all fragment ions were obtained as bar-graph lists, and the average intensities of five measurements, if not otherwise mentioned, were input to a PC-9801 VM2 personal computer and calculated as reported.¹⁾ The ion groups for calculating the fitness indices were selected according to their ion intensities at the following five levels: all ions with intensities of more than 0.1% ($>0.1\%$), of more than 1% ($>1\%$), of more than 3% ($>3\%$), of more than 5% ($>5\%$) and of more than 10% ($>10\%$).

The mass spectral list of the commonly observable ions throughout all the isomers and the very characteristic ions of each isomer, are shown in Table I with those of the natural compound. 1-Me differed from the others by the presence of characteristic fragment ions such as m/z 116 and m/z 89. All the other isomers gave almost identical mass spectra, except for additional differences of intensity at m/z 130 and m/z 131 for 2-Me and 3-Me. Two isomers (2-Me and 3-Me) gave the base ion at m/z 130, and 1-Me at m/z 131, whereas the base ions of the isomers whose methyl groups were situated at the benzene ring fluctuated between at m/z 130 and at m/z 131. Three isomers (4-Me, 6-Me and 7-Me) gave it once each at m/z 130, and 5-Me twice within 5 independent measurements.

The fitness indices of all the isomers calculated at 5 ion-intensity levels are summarized in Table II. At all levels, the index value of 3-Me was the smallest among the isomers, the results suggesting 3-Me as the most probable isomer for natural methylindole. Statistical significance was observed in all results between 3-Me and the runner-up (20.0% and 31.3% calculated from 28 ions, 15.3% and 22.5% from 22 ions, 7.7% and 22.2% from 10 ions, and 6.2% and 24.4% from 8 ions) at a 99% confidence level (t -test), except for the case of the ion group with an intensity of more than 10% (3.3% and 24.3% from 3 ions). When the total numbers of ions used became lower as the intensity level increased, the fitness indices became smaller and statistically less significant, as already reported.¹⁾

As a result, skatole of *C. gallicola* was demonstrated to be identifiable among the methylindoles by an application of the fitness index method. When a similar calculation

* All the isomers of methylindoles are abbreviated as 1-Me, 2-Me, and so on, indicating the substituted position of a methyl group.

TABLE I. LIST OF FRAGMENT IONS AND THEIR INTENSITIES FOR ALL ISOMERS

<i>m/z</i>	1-Me	2-Me	3-Me	4-Me	5-Me	6-Me	7-Me	Natural indole
133	0.3	0.5	0.2	0.3	0.4	0.4	0.6	0.3
132	9.5	9.3	6.3	8.6	8.6	9.9	10.9	5.9
131	100.0	97.5	69.8	99.9	99.4	99.5	99.8	73.4
130	59.9	100.0	100.0	95.4	98.2	98.6	96.5	100.0
129	1.4	2.1	2.4	1.8	2.4	1.9	1.8	2.6
128	1.9	2.4	2.3	2.5	2.4	2.2	2.5	2.4
116 ^a	6.4	0	0	0	0	0.1	0.4	0
104	1.4	2.6	1.8	3.1	2.7	2.9	2.8	1.9
103	6.8	9.4	8.9	8.2	8.5	9.4	8.4	9.1
102	2.9	4.0	5.7	4.7	4.5	5.6	4.9	5.2
101	1.1	1.0	1.1	0.9	1.6	1.1	1.1	1.1
90	6.3	1.9	0.4	0.3	0.2	0.2	0.4	0.3
89	11.8	2.9	0.9	1.4	1.2	1.3	1.6	0.9
87	1.1	0.7	0.4	0.9	0.9	0.5	1.1	0.3
78	1.5	1.7	2.0	2.4	2.6	2.9	3.4	2.1
77	10.2	13.7	14.6	15.0	14.4	15.6	16.0	14.9
76	1.6	1.9	2.3	2.5	2.5	2.2	2.8	2.4
75	2.0	2.1	2.5	2.0	2.4	2.0	2.0	2.5
74	1.7	1.2	1.1	1.7	1.8	1.3	1.7	2.0
66	5.4	4.8	4.1	4.2	4.0	4.5	4.7	3.8
65	2.6	5.7	6.0	5.8	5.1	5.9	6.0	6.3
64	1.8	2.1	1.6	1.6	1.6	1.6	2.3	1.3
63	6.9	5.2	2.7	3.6	3.7	3.9	3.7	3.0
62	2.7	2.2	1.1	2.1	1.6	1.9	1.7	1.4
61	0.8	0.7	0.4	0.4	0.4	0.5	0.5	0.3
53	0.3	1.2	0.5	1.2	0.8	0.8	1.0	0.6
52	1.6	2.8	3.0	3.8	4.2	4.0	3.9	2.7
51	5.2	5.9	7.5	7.4	8.6	7.8	7.0	7.3
50	2.6	3.1	3.2	2.9	3.2	3.6	3.7	2.9
Av. CV%	18.96	19.42	21.41	22.00	19.05	15.06	19.91	17.60

^a This data was listed for its characteristics, although it was unusable for calculating the fitness indices.

TABLE II. CALCULATED RESULTS OF NATURAL METHYLINDOLE AGAINST ALL METHYLINDOLES AT VARIOUS CONDITIONS

Selected intensity	Ion No.	Methylindoles						
		1-Me	2-Me	3-Me	4-Me	5-Me	6-Me	7-Me
> 0.1%	28	226.1	70.6	<u>20.0^a</u>	34.6	31.3	31.4	42.5
> 1%	22	53.0	33.8	<u>15.3^a</u>	26.7	22.5	29.1	30.8
> 3%	10	78.1	36.2	<u>7.7^a</u>	22.2	25.4	22.6	29.0
> 5%	8	79.8	35.4	<u>6.2^a</u>	24.4	29.1	25.0	32.4
> 10%	3	79.7	28.7	<u>3.3^b</u>	27.7	26.5	24.3	25.9

Underlined results indicate the greatest similarity to natural methylindole.

^a Significant at 99% confidence level (*t*-test); ^b at 95% confidence level.

was applied to the other isomers by hypothesizing a single result of mass measurement as the value of the natural isomer, 1-Me and 2-Me also suggested the correct cor-

responding isomer; however, with the other 4 isomers whose methyl group is situated in the benzene ring, the method was not always reliable.

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