[Chem. Pharm. Bull. 32(1) 325—331 (1984)]

Plant Constituents Biologically Active to Insects. IV.[1] Antifeedants for the Larvae of the Yellow Butterfly, *Eurema hecabe mandarina*, in *Arachniodes standishii*

ATSUSHI NUMATA,*,[a] TAKAKO KATSUNO,[a] KYOKO YAMAMOTO,[a] TOMOKO NISHIDA,[a] TSURUKO TAKEMURA,[a] and KO SETO[b]

Osaka College of Pharmacy, 2–10–65 Kawai Matsubara, Osaka 580, Japan[a] and Osaka Museum of Natural History, 1–23 Nagaikoen Higashisumiyoshi, Osaka 546, Japan[b]

(Received May 12, 1983)

Nine species of plants having antifeeding activities for the larvae of the yellow butterfly, *Eurema hecabe mandarina* DE L'ORZA, were found. From one of them, *Arachniodes standishii* (MOORE) OHWI, the known compound, 1-(2,4,6-trimethoxyphenyl)but-*trans*-2-en-1-one (I), was isolated as an antifeedant. This compound was synthesized according to the Friedel–Crafts procedure, which also gave two new compounds, 1,3-bis(2,4,6-trimethoxyphenyl)butan-1-one (VI) and 1-(2,4,6-trimethoxyphenyl)-3-[3-(2-*trans*-butenoyl)-2,4,6-trimethoxyphenyl]butan-1-one (VII), as by-products. Treatment of 1,3,5-trimethoxybenzene (V) with ferric chloride was found to afford 2,2′,4,4′,6,6′-hexamethoxybiphenyl (VIII), which has been synthesized according to Ullmann's procedure.

Keywords——*Eurema hecabe mandarina*; *Arachniodes standishii*; antifeedant; 1-phenylbutan-1-one derivative; 1-phenylbut-*trans*-2-en-1-one derivative; biphenyl; Aspidiaceae

Previously we investigated feeding stimulants,[1,2] attractants,[3] and arrestants[3] for the larvae of the yellow butterfly, *Eurema hecabe mandarina* DE L' ORZA, in their host plant, *Lespedeza cuneata* G. DON, in relation to host selection. This paper reports a survey of various plants having antifeeding activities against the larvae of the yellow butterfly, and the isolation and synthesis of 1-(2,4,6-trimethoxyphenyl)but-*trans*-2-en-1-one (I) as an antifeedant from one of the antifeeding-active plants, *Arachniodes standishii* (MOORE) OHWI (Japanese name, Ryomenshida).

Results and Discussion

Antifeeding activities of *E. hecabe mandarina* larvae to various plants were tested according to the bioassay procedure described in "Experimental." Table I shows that nine species of plants have antifeeding activities. Antifeedants in two of them, *Osmunda japonica* THUNB. and *Lycoris radiata* HERB., have been briefly reported in preliminary communications.[4,5] This time, *A. standishii* was used as a source material for the isolation of antifeedants.

The dried whole plant was extracted successively with hexane, ether, and methanol. An active principle was separated from the extracts by using various chromatographic techniques with monitoring for antifeeding activity (Chart 1). The hexane and ether extracts were found to have feeding inhibitory activities. The former was fractionated into basic, acidic, phenolic, and neutral fractions in the usual way. The main constituent in the active neutral fraction was expected from the proton nuclear magnetic resonance (^{1}H NMR) spectrum to be a mixture of hydrocarbons. The isolation of antifeedants from this fraction was not attempted because

TABLE I. Antifeeding Activities of Various Plants

Family	Species[a)]	Mean frass[b)] count	Feeding ratio (%)
Aspidiaceae	*Arachniodes standishii* (MOORE) OHWI	6.8±0.8	20.8
	Dryopteris pacifica (NAKAI) TAGAWA	8.0±1.9	26.6
	Dryopteris bissentiana (BAK.) C. CHR.	7.4±1.3	23.7
Blechnaceae	*Struthiopteris niponica* (KUNZE) NAKAI	7.0±2.8	21.7
Athyriaceae	*Onoclea orientalis* HOOK.	6.4±3.0	18.8
Adiantaceae	*Adiantum pedatum* L.	7.0±0.7	21.7
Osmundaceae	*Osmunda japonica* THUNB.	4.8±0.8	11.1
Dennstaedtiaceae	*Pteridium aquilinum* KUHN	8.8±0.8	30.4
Thelypteridaceae	*Thelypteris acuminata* (HOUTT.) MORTON	19.2±7.5	80.7
Amaryllidaceae	*Lycoris radiata* HERB.	9.8±1.3	35.3
Ulmaceae	*Celtis sinensis* PERS. var. *japonica* NAKAI	15.0±2.1	60.4
Moraceae	*Morus bombycis* KOIDZ.	13.2±4.6	51.7
Compositae	*Artemisia vulgaris* L. var. *indica* MAXIM.	13.3±2.2	52.2
	Cirsium japonicum DC.	14.3±2.2	57.0

a) The concentration of the plants for the test was 7.7%.
b) The mean frass counts of the basal diet and the control diet were 2.5±0.4 and 23.2±0.6, respectively.

separation of the hydrocarbons seemed to be difficult. Another active extract was partitioned between chloroform and water, and the active chloroform-soluble fraction was chromatographed successively on LH-20 and silicic acid to give the active fraction 4. As it was expected from the infrared (IR) spectrum that fatty acids were contained in the fraction, it was fractionated into acidic and other fractions. Since the acidic fraction showed low antifeedant activity, the other fraction was further subjected to silicic acid column chromatography to yield a pure active compound (I).

The active compound (I), mp 90—91 °C, was assigned the molecular formula $C_{13}H_{16}O_4$ on the basis of the mass spectrum (MS) (M^+: m/z 236.1028). The ultraviolet (UV), IR, and ^1H NMR spectra showed I to be 1-(2,4,6-trimethoxyphenyl)but-*trans*-2-en-1-one, which has been isolated from the same plant by Murakami *et al.*[6] The antifeeding activities of this compound are shown in Table II, which indicates that the limiting concentration for activity is 0.01%.

Purification of the methanol extract by partitioning, followed by a combination of LH-20 and silicic acid column chromatography, gave sucrose (II), β-sitosterol (III) and β-sitosterol D-glucoside (IV), together with a small amount of I. The former two compounds (II and III) were identified by comparison of their spectral data and gas liquid chromatography (GLC) behavior with those of authentic samples. The last compound (IV) was identified by GLC analysis of the acid hydrolysis products.

Since I has never been synthesized, we investigated its synthesis. The Friedel–Crafts reaction of 1,3,5-trimethoxybenzene (V) with crotonyl chloride in the presence of aluminum chloride in boiling carbon disulfide did not afford the desired I, but gave compound VI as a main product (Chart 2). Its structure was established from the spectral data, as shown in Table III, to be 1,3-bis(2,4,6-trimethoxyphenyl)butan-1-one. The ^1H NMR spectral data in

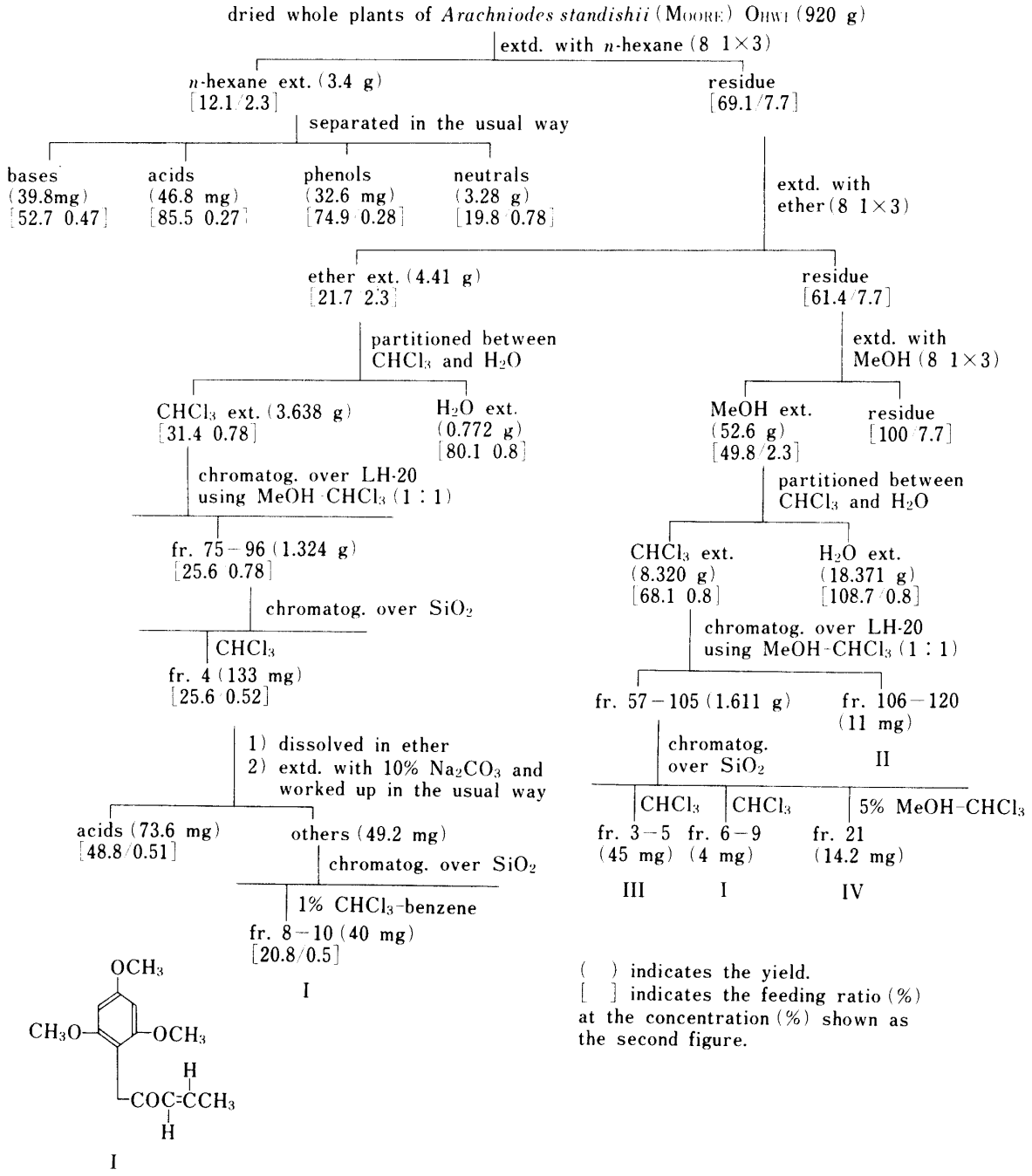


Chart 1

TABLE II. Antifeeding Activities of I

Sample	Concentration (%)	Mean frass count	Feeding ratio (%)
Basal diet		2.5±0.4	
Control diet		23.2±0.6	
I	0.1	10.0±1.9	36.2
	0.01	9.6±2.7	34.3
	0.001	19.4±7.2	81.6

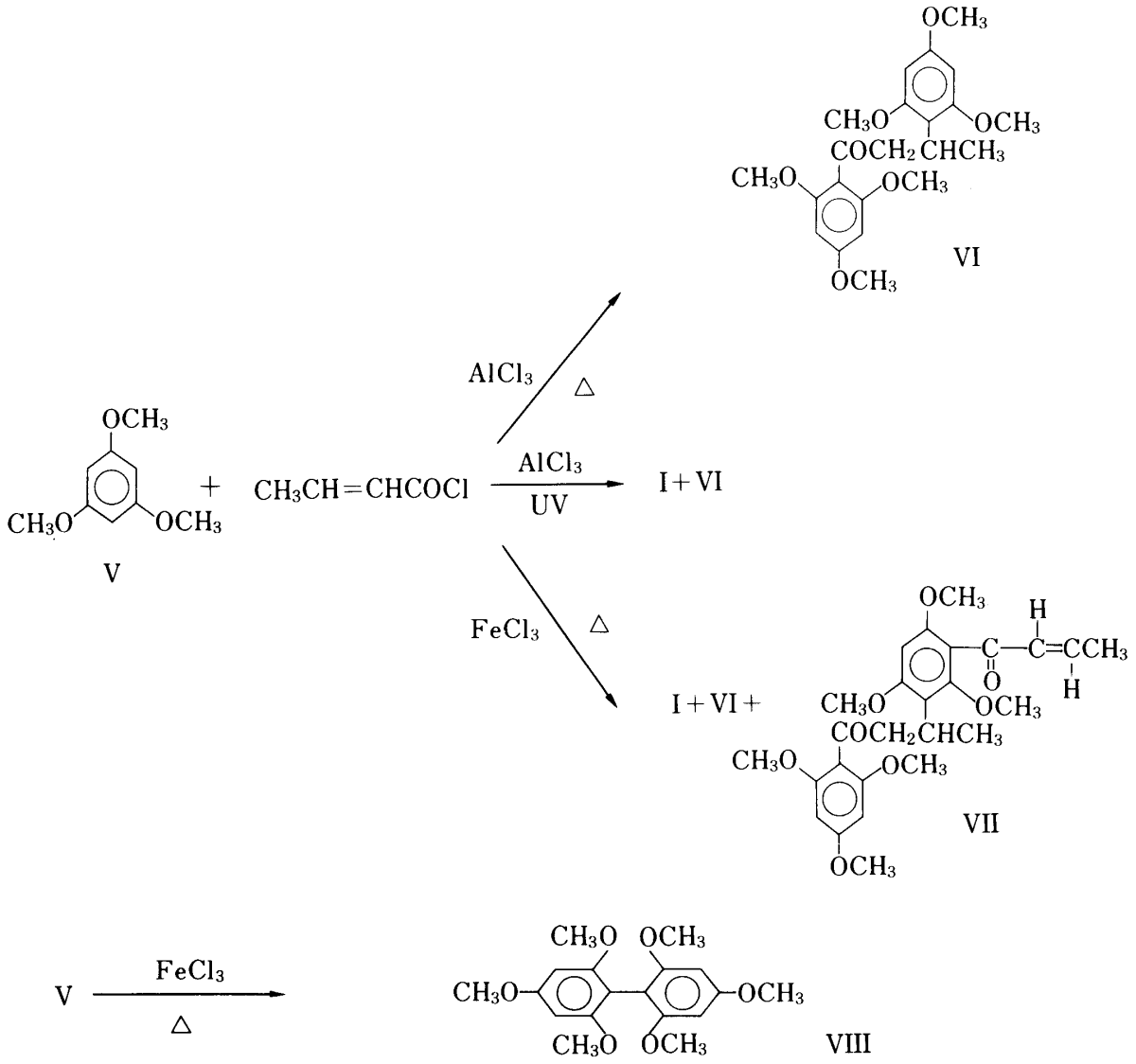


Chart 2

Table III were assigned by comparison with those of I, V, and VI or VII.

The foregoing reaction at room temperature for 1 h under exposure to the rays of a mercury vapor lamp afforded I and VI in very low yields. When the same reaction was carried out for 3 h, none of the three compounds, I, VI, and VII (described below), were obtained. Next, this Friedel–Crafts reaction was conducted in boiling carbon disulfide for 4 h, using ferric chloride as a catalyst, to give I, VI, and VII in 16, 0.4, and 1% yields, respectively. The structure of VII was established from the spectral data (Table III) to be 1-(2,4,6-trimethoxyphenyl)-3-[3-(2-*trans*-butenoyl)-2,4,6-trimethoxyphenyl]butan-1-one. The natural product (I) was identical with the compound (I) synthesized herein.

In the course of the synthesis of I described above, we noticed that heating of V in the presence of ferric chloride in carbon disulfide gave a biphenyl. The product was confirmed by spectral analysis to be 2,2′,4,4′,6,6′-hexamethoxy-1,1′-biphenyl, which has been prepared according to Ullmann's procedure.[7]

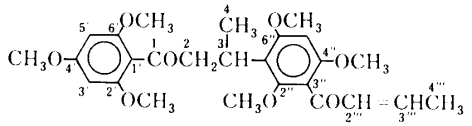
Experimental

Materials——i) Plants: All test plants were collected in the fields near Kawachinagano city, Osaka. Dried

TABLE III. ^{1}H Chemical Shifts (δ ppm) of 1-Phenylbutan-1-one Derivatives in $CDCl_3$

Proton	I	VI	VII[a]
2-H	6.30 (1H, d, J=16 Hz)	3.10 (1H, dd, J=7, 17 Hz) 3.22 (1H, dd, J=8, 17 Hz)	3.07 (1H, dd, J=5, 16 Hz) 3.23 (1H, dd, J=10, 16 Hz)
3-H	6.62 (1H, qd, J=7, 16 Hz)	3.97 (1H, quintet of d, J=7, 8 Hz)	4.02 (1H, m)
3-CH_3	1.88 (3H, d, J=7 Hz)	1.23 (3H, d, J=7 Hz)	1.29 (3H, d, J=7 Hz)
2′-OCH_3 6′-OCH_3	3.73 (6H, s)	3.71 (6H, s)	3.72 (6H, s)
4′-OCH_3	3.81 (3H, s)	3.76 (3H, s)[b]	3.77 (3H, s)[b]
3′-H 5′-H	6.11 (2H, s)	6.06 (2H, s)	6.06 (2H, s)
2″-OCH_3 6″-OCH_3		3.67 (6H, s)	3.62 (3H, s) 3.80 (3H, s)
3″-H 5″-H		6.03 (2H, s)	6.22 (1H, s)
4″-OCH_3		3.78 (3H, s)[b]	3.74 (3H, s)[b]
2‴-H			6.35 (1H, d, J=17 Hz)
3‴-H			6.61 (1H, qd, J=7, 17 Hz)
3‴-CH_3			1.89 (3H, d, J=7 Hz)

a) Numbering of VII:



b) The indicated assignments for each compound may be interchanged.

bulbs of *L. radiata* and dried leaves of other species of plants were used for the test. *L. cuneata* was grown in our medicobotanical garden.

ii) Insects: Adult yellow butterflies, collected in the field of Kawachinagano city, were allowed to lay their eggs and the 5th instar larvae, reared in our laboratory as described in the previous paper,[2] were used for the present experiments.

Bioassay Procedure——Five larvae, starved for 4 h, were placed on each of the following diets; (1) the basal diet (1% agar only); (2) the control diet prepared by incorporating dried leaf powder of *L. cuneata* (0.39 g) in 1.4 ml of 1% agar; (3) the test diets prepared by incorporating plant powders or fractions into dried leaf powder of *L. cuneata* in 1 ml of 1% agar at a total weight of 1.28 g. The number of frass pellets deposited during the test period (20 h) was then counted. The feeding inhibitory activities to the various test diets were evaluated on the basis of the feeding ratio calculated as follows:

$$\text{feeding ratio} = \frac{\text{the mean frass count for a test diet} - \text{the mean frass count for the basal diet}}{\text{the mean frass count for the control diet} - \text{the mean frass count for the basal diet}}$$

Feeding inhibitory activities were appraised as positive when the feeding ratio was less than 50%, and were considered to be strong at 0—20% feeding ratio and to be slight at 20—50%.

Instruments——All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The UV spectra were recorded with a Hitachi 323 spectrophotometer and the IR spectra with a Hitachi EPI-G2 spectrometer. The ^{1}H NMR spectra were taken on a Hitachi R 40 spectrometer (90 MHz) and the carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra on a Hitachi R 26 (10 MHz). Chemical shifts are given in parts per million (δ) down-field from tetramethylsilane (TMS) as an internal standard. The MS were taken on a Hitachi M 80 spectrometer. GLC was run on a Hitachi 063 equipped with a flame ionization detector, according to the procedure described in the previous paper.[2]

Extraction and Fractionation——The whole plants of *A. standishii* were extracted with several solvents and the resulting extracts were fractionated as shown in Chart 1.

1-(2,4,6-Trimethoxyphenyl)but-*trans*-2-en-1-one (I)——Colorless plates, mp 91—92 °C (from *n*-hexane–acetone). High resolution MS m/z: Found 236.1028. Calcd for $C_{13}H_{16}O_4$ (M^+), 236.1047. UV λ_{max}^{MeOH} nm (log ε): 224 (4.20), 302

(3.43). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1641 (C=C–CO–Ph), 1602, 1588 (Ar. ring). MS *m/z* (relative intensity %): 236 (M^+, 92), 221 (90), 219 (69), 208 (63), 195 [$(MeO)_3$ $PhCO^+$, 100], 181 (89). The spectral data were in accord with the published values[6] and those of the synthetic sample.

Sucrose (II)——Colorless prisms, mp 168—170 °C (from EtOH–H_2O). It was identified by comparison of the ^{13}C NMR spectrum and GLC with those of an authentic sample.

***β*-Sitosterol (III)**——Colorless needles, mp 137—139 °C (from $CHCl_3$–EtOH). It was identified by comparison of the MS and GLC with those of an authentic sample.

***β*-Sitosterol D-Glucoside (IV)**——Colorless needles, mp 295—300 °C (from EtOH). The tetraacetate, mp 165—168 °C (from $CHCl_3$–MeOH). It was identified by GLC comparison of the acid hydrolysis products with authentic samples.

Reaction of 1,3,5-Trimethoxybenzene (V) with Crotonyl Chloride Using Aluminum Chloride as a Catalyst——i) Aluminum chloride (1.5 g) and V (0.8 g) were added to a solution of crotonyl chloride (1.5 g) in carbon disulfide (30 ml). The mixture was heated under reflux for 4 h, and then concentrated under reduced pressure. The residue was diluted with water, and the solution was extracted with chloroform. The chloroform layer was washed with 10% aqueous sodium carbonate, then with water, and evaporated to dryness. The residue (1.05 g) was purified by alumina column chromatography using a chloroform–benzene gradient as the eluent. The initial fraction eluted with benzene gave the starting material (283 mg), and the later fraction, eluted with 10% chloroform–benzene, afforded 1,3-bis(2,4,6-trimethoxyphenyl)butan-1-one (VI) (176.7 mg, 10% yield) as colorless needles, mp 76—80 °C (from acetone). *Anal.* Calcd for $C_{22}H_{28}O_7$: C, 65.33; H, 6.98. Found: C, 65.38; H, 7.01. UV λ_{max}^{MeOH} nm (log ε): 225 (sh) (4.31), 276 (3.83). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1677 (PhCO), 1600, 1586 (Ar. ring). MS *m/z* (%): 404 (M^+, 46), 236 (75), 221 (32), 208 (51), 195 [$(MeO)_3PhCO^+$, 100], 180 (24), 168 (25). ii) Aluminum chloride (1.5 g) was added to a solution of crotonyl chloride (1.5 g) in carbon disulfide (30 ml). With vigorous stirring, a solution of V (0.8 g) in carbon disulfide (10 ml) was added to the mixture cooled to 0 °C. The solution was exposed to the rays of a mercury vapor lamp (100 W) for 1 h at room temperature. The solution was then poured into iced hydrochloric acid solution, and extracted with chloroform. The chloroform solution was washed with 10% aqueous sodium carbonate, dried over sodium sulfate, and evaporated. The residue (0.8 g) was purified by repeated alumina column chromatography using a chloroform–benzene gradient as the eluent. Elution with 2% chloroform–benzene gave, together with VI (23 mg, 1% yield), a small amount of I (11 mg, 1% yield) as colorless plates, mp 91—92 °C (from *n*-hexane–acetone). *Anal.* Calcd for $C_{13}H_{16}O_4$: C, 66.08; H, 6.83. Found: C, 65.95; H, 6.59.

Reaction of V with Crotonyl Chloriode Using Ferric Chloride as a Catalyst——Ferric chloride (1.5 g) and V (0.8 g) were added to a solution of crotonyl chloride (1.5 g) in carbon disulfide (30 ml). The mixture was heated under reflux for 4 h, and then concentrated *in vacuo*. The residue was diluted with water and extracted with chloroform. The chloroform solution was washed with 10% aqueous sodium carbonate and water, and then evaporated. The oily residue (1.2 g) was chromatographed over alumina using a chloroform–benzene gradient as the eluent. The earlier fraction eluted with benzene gave I (176.9 mg, 16% yield) as colorless plates. Further elution with 10% chloroform–benzene gave VI (8 mg, 0.4% yield). The later fraction eluted with 50% chloroform–benzene afforded 1-(2,4,6-trimethoxyphenyl)-3-[3-(2-*trans*-butenoyl)-2,4,6-trimethoxyphenyl]butan-1-one (VII) (22.8 mg, 1% yield) as a colorless oil. UV λ_{max}^{MeOH} nm (log ε): 224 (4.45), 279 (3.90). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1679 (PhCO), 1650 (C=C–CO), 1597 (Ar. ring). MS *m/z* (%): 472 (M^+, 6), 321 (57), 249 (37), 221 (24), 207 (36), 195 [$(MeO)_3PhCO^+$, 100].

Treatment of V with Ferric Chloride——Ferric chloride (1.5 g) was added to a solution of V in carbon disulfide (30 ml), and the mixture was heated under reflux for 4 h, then concentrated *in vacuo*. The residue was diluted with water and extracted with chloroform. The chloroform solution was washed and evaporated. The oily residue (0.79 g) was purified by alumina column chromatography using an ethyl acetate–benzene gradient as the eluent. The initial fraction eluted with benzene gave the starting material (127 mg). Elution with 5% ethyl acetate–benzene gave 2,2′,4,4′,6,6′-hexamethoxy-1,1′-biphenyl (VIII) (44 mg, 3% yield) as colorless plates (from *n*-hexane–acetone), mp 156—158 °C (lit.[7] mp 156 °C).

Acknowledgement The authors are very grateful to Prof. S. Matsunaga, Miss M. Kurimoto, and Mrs. Y. Tsukamoto of this college for the MS measurements and elemental analyses.

References and Notes

1) *a*) Part III of this series: A. Numata, K. Hokimoto, H. Yamaguchi, and H. Nishida, *Appl. Entomol. Zool.*, **17**, 582 (1982); *b*) Part II: A. Numata, K. Hokimoto, T. Takemura, S. Matsunaga, and R. Morita, *Chem. Pharm. Bull.*, **31**, 436 (1983).
2) A. Numata, K. Hokimoto, A. Shimada, H. Yamaguchi, and K. Takaishi, *Chem. Pharm. Bull.*, **27**, 602 (1979).
3) A. Numata, K. Hokimoto, S. Kuwana, K. Tsutsumi, H. Yamaguchi, and K. Takaishi, Abstract of Papers, 98th Annual Meeting of Pharmaceutical Society of Japan, Okayama, 1978, p. 315.
4) A. Numata, K. Hokimoto, T. Takemura, and S. Fukui, *Appl. Entomol. Zool.*, **18**, 129 (1983).

5) A. Numata, T. Takemura, H. Ohbayashi, T. Katsuno, K. Yamamoto, K. Sato, S. Kobayashi, *Chem. Pharm. Bull.*, **31**, 2146 (1983).
6) N. Tanaka, H. Maehashi, S. Saito, T. Murakami, Y. Saiki, C.-M. Chen, and Y. Iitaka, *Chem. Pharm. Bull.*, **28**, 3070 (1980).
7) W. Riedl, *Justus Liebigs Ann. Chem.*, **597**, 148 (1955).