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FRAGRANT AND BIOLOGICALLY ACTIVE CONSTITUENTS OF THE CITRUS CULTIVAR JYABON

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Key Word Index—*Citrus*, Rutaceae, peel; jyabon, essential oil; psoralen, coumarins, flavanes, *β*-myrcene, *Unaspis yanonensis*, *Oxycetonia jucunda*.

Abstract—1,8-Cineol is probably a key material for the fragrance characteristic of the fruit of jyabon. Further, the peels of this citrus contain psoralen which has pesticidal activity against *Unaspis yanonensis* (arrowhead scale insect). The flowers contain a large amount of *β*-myrcene which attracts *Oxycetonia jucunda* (citrus flower chafer). The composition of essential oil constituents of the leaves is similar to that of *Citrus grandis* var anseikan.

INTRODUCTION

The peels and fruit juice of the citrus cultivar jyabon possess a delicate fragrance different from that of fruits of other citrus cultivated in Japan. In addition, we recently observed that the arrowhead scale insect, *Unaspis yanonensis* Kuwana, is not parasitic on the fruits of jyabon, even when this plant is cultivated with citrus plants which are parasitized by this insect. Further, the flowers of jyabon diffuse a sweet aroma and attract the citrus flower chafer, *Oxycetonia jucunda* Faldermann. We have now examined the fragrant and biologically active constituents responsible for these observed phenomena

RESULTS AND DISCUSSION

Fragrant constituents in the fruits of jyabon

The essential oils obtained from the peels and fruit juice of jyabon by steam distillation were analysed by GC and GC/MS The essential oil of the peels (Table 1) is mainly composed of limonene and *β*-myrcene; the oil of the fruit juice is mainly α-terpineol and 1,8-cineol Both oils develop the aroma similar to that of the juice obtained from the whole cold-pressed fruit. The essential oil of the peels was subjected to silica gel column chromatography to give fraction (A) possessing a fragrance characteristic of the fruit of jyabon and fraction (B) possessing a fragrance differing from that of the fraction (A). Fraction (A) contains 1,8-cineol (22.2%), citronellal (12.9%), neral (11 6%) and *cis*-carveol (11 1%), while fraction (B) has α-terpineol (35.2%) and linalool (16.1%). The fragrance characteristic of fraction (A) is 1,8-cineol, which is lost when the oil is left at room temperature for 10 hr, leaving behind citronellal (16.5%), neral (14.9%) and *cis*-carveol (14.3%). None of the following peel oils have the same fragrance: *Poncirus trifoliata* Rafin., *Citrus limon* Burm. f., *C. aurantifolia* Swingle, *C. grandis* var. anseikan Hort. ex Tanaka, *C. natsudaidai* Hayata, *C. junos* Sieb. ex Tanaka, *C. sphaerocarpa* Hort. ex Tanaka, *C. sudachi* Hort. ex Shirai, *C. unshiu* Marc. and *Fortunella japonica* Swingle Thus, 1,8-cineol is a key material for the fragrance characteristic of the fruit of jyabon

Constituents having pesticide activity against a scale insect

Four non-volatile constituents, aurapten (**1**), psoralen (**2**), hesperidin (**3**) and naringin (**4**), were isolated, in addition to the essential oil, from the peels of jyabon. The growth inhibition activity of these non-volatile constituents and the essential oil against the arrowhead scale insect was tested by use of a segment of the leaf of *C. unshiu*. Figure 1 shows the body length and body width of the scale insect, after living for three weeks on the segment of the leaf placed on an agar medium including each of the constituents. Larvae of the arrowhead scale insect develop into adults through two stages. In the first stage, hatched walking-larvae adhere to a citrus leaf and their sexes are still indistinguishable, but in the second stage, the adhered larvae differ: the females secrete a scale material and the males a cottony material [3]. Psoralen was strongly inhibitory to the growth of the females, whereas it showed only weak activity against the males. In addition, all the females died at the first stage and *ca* 14% of the males died at the second stage after breeding on the segment of the leaf (Table 2).

Finally, the distribution of the four non-volatile constituents (**1**)–(**4**) was examined in the peels of the 10 selected citrus by HPLC (Table 3). Psoralen was found to be specific to the peels of jyabon This is the first isolation of psoralen from citrus, though the related bergaptol, bergapten, isopimpinellin, imperatorin and isoimperatorin have all been described from this genus [4].

Attractants for the citrus flower chafer

The composition and attractancy of volatile constituents of the whole flowers and their organs, such as petals, pistils, stamens and sepals, of jyabon were compared with those of the volatile constituents of *C. unshiu*; the flowers of *C. unshiu* were only weakly attractive to the

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Table 1 Composition (%) of the essential oil constituents from the peels and fruit juice of jyabon

Compounds	Peels	Juice	Refs*
Monoterpenes†‡	99.3	100	
Hydrocarbons	97.5	17.6	
α-Pinene	0.2	1.3	
Camphene	0.2		
β-Pinene	1.1	-	
β-Myrcene	17.3	--	[1]
1,8-Cineol	tr	12.1	
Limonene	78.2	6.1	
α-Phellandrene	---	–	[1]
γ-Terpinene	0.5	4.0	[1]
Terpinolene		1.1	[1]
Oxygenated compds	1.8	82.5	
Linalool	0.3	2.0	
Limonene-1,2-epoxide	tr	2.3	
Citronellal	0.3	2.4	[1]
α-Terpinyl acetate	–	7.1	
Terpinen-4-ol	-	0.6	
α-Terpineol	0.2	39.1	
trans-Carveol	0.4	3.5	
cis-Carveol	tr	–	
Nerol	0.1	0.5	
Neral	0.4	0.7	
Geraniol	-	0.6	
Geranial	–	0.4	
Neryl acetate	---	4.4	
Geranyl acetate	0.1	0.4	
Sesquiterpenes†‡	0.7	—	
β-Bisabolene	0.4		[2]
α-Humulene	0.1		[2]
α-Elemene	0.2	—	[2]

* References for identification of the constituents

† The figures include percentages of the other unidentified compounds

‡ Described in the order of elution from an OV-101 GC column.

tr trace (<0.1%)

citrus flower chafer The composition of the hexane-soluble constituents from the flowers and other organs of jyabon are given in Table 4. The constituents of the whole flowers and the petals, pistils and sepals are monoterpene hydrocarbons, such as β-pinene, β-myrcene, limonene and α-phellandrene, whereas the stamens contain methyl laurate, methyl myristate and methyl palmitate

The attractancy was tested by use of the olfactometer [5]. Attraction rates of hexane extracts from the whole flowers of jyabon, *C. unshiu* and their organs and the rates of major constituents in the hexane extract from the petals of jyabon for *O jucunda* are given in Table 5 Attractancy at the concentration of 1.0 ppm was in the order of pistils (attraction rate, 87.9%), petals (79.2%), whole flowers (58.7%), sepals (47.8%) and stamens (22.5%) The attractancy of jyabon was markedly higher than that of the whole flowers of *C. unshiu* and their different parts So, the hexane-soluble constituents of the whole flowers and organs of jyabon (Table 4) was com-

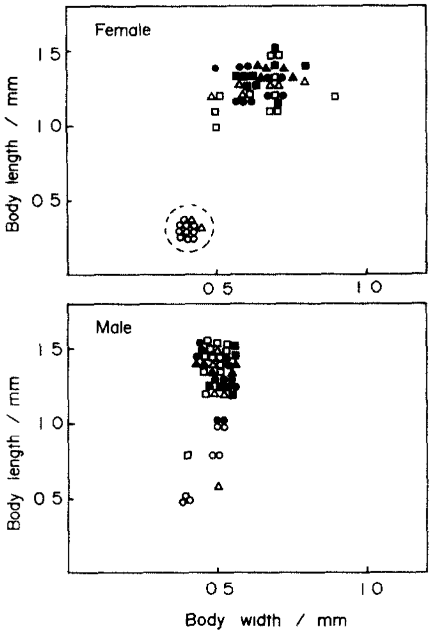

Fig 1 Body length and body width of *U. yanonensis* after breeding for three weeks on the segment of the leaf placed on 0.5% agar medium including essential oil (▲), aurapten (△), psoralen (○), hesperidin (●) or naringin (□) (■), control Insect group enclosed with dotted line was in the first stage

pared with those of the corresponding organs of *C. unshiu*, and a large amount of β-myrcene was found to be specifically present in the petals and pistils of jyabon This hydrocarbon is strongly attractive (76.4%) at the concentration of 0.1 ppm (Table 5) Figure 2 shows the relationship (solid line) between the absolute amounts of β-myrcene present in the solns (1.0 ppm) of the hexane extracts from the whole flowers and their organs and the attraction rates of the hexane extracts and the relationship (dotted line) between the absolute amounts of the authentic hydrocarbon and their attraction rates. Two curves have a maximum at *ca* 10.0 μg and their shapes almost coincide Thus, β-myrcene is one of the most potent constituents which attract the citrus flower chafer to the flowers of jyabon. However, the attraction rates of the authentic hydrocarbon were slightly lower than those of the hexane extracts (Fig 2) This is due to the presence of other minor attractants, in addition to β-myrcene.

Comparison of the essential oil compositions of jyabon with those of the 10 selected citrus

Since jyabon is said to be a chance seedling, the essential oil compositions in the leaves of jyabon were compared with those in leaves of 10 selected citrus Two kinds of leaves of jyabon, i e. the new leaves and the old leaves from the year before, were collected in May The essential oils of both the new and old leaves (Table 6) are mainly composed of monoterpene hydrocarbons, such as β-pinene, β-myrcene and α-phellandrene and sesquiterpene hydrocarbons, such as caryophyllene, β-bisabolene and α-elemene The new and old leaves differ slightly in content of oxygenated monoterpenes, such as linalool, neral and geraniol After statistical treatment [6]

Table 2. Death rate of *U. yanonensis* on the segment of the leaf at the 1st and 2nd stage by administrations of essential oil (ES), aurapten (1), psoralen (2), hesperidin (3) and naringin (4)

Insect (larva)		Death rate (survivors rate)/%					
		ES	1	2	3	4	control
1st stage*		18 8 (81 2†)	28.0 (72 0)	61 3 (38 7)	1 1 (98 9)	7 1 (92.9)	9 4 (90 6)
2nd stage	female	0 (44.0)	0 (48.0)	0 (14 3)	0 (51 9)	0 (47 5)	0 (50.5)
	male	0 (37 2)	0 (24.0)	0 (24.4)	0 (47 0)	0 (45 4)	0 (40 1)

* The females and the males of the larvae were indistinguishable in the 1st stage.

† All the survivors in the 1st stage grew up to the second stage larvae

Table 3. Distribution of aurapten (1), psoralen (2), hesperidin (3) and naringin (4) in the peels of jyabon and 10 selected citrus

Citrus	1	2	3	4
Jyabon	+*	+	+	+
Poncirus trifoliata Rafin.	+	−*	−	−
Citrus limon Burm. f	−	−	+	+
C aurantifolia Swingle	−	−	+	+
C grandis var anseikan Hort ex Tanaka	+	−	−	+
C natsudaidai Hayata	+	−	+	+
C junos Sieb ex Tanaka	+	−	+	+
C sphaerocarpa Hort. ex Tanaka	+	−	−	+
C. sudachi Hort. ex Shirai	−	−	+	+
C unshiu Marc.	−	−	−	−
Fortunella japonica Swingle	+	−	−	−

* + and − denote present and absent, respectively

and following Hanshu's theory [7], correlation coefficients of jyabon to the 10 selected citrus were determined (Table 7). The highest correlation was observed between jyabon and *C grandis* var. anseikan. Further, variation in the correlation coefficients of jyabon to the 10 selected citrus during May to November is shown in Fig. 3. The highest correlations are between jyabon and *C. grandis* var. anseikan for the new and old leaves in all months, indicating a genealogically close relationship between jyabon and *C. grandis* var anseikan.

EXPERIMENTAL

General. Analytical and prep. TLC were carried out on Merck 60 GF_{254} silica gel plates (thickness. 0.25 and 0.75 mm, respectively). GC and co-GC with authentic samples were performed on an instrument equipped with an FID and a glass capillary column (WCOT, 0.20 mm × 50 m) coated with OV-101 (thickness: 0.25 μm) by programming the column temp. at 2°/min from 40 to 260° with N_2 gas as carrier GC/MS analyses were performed on a Shimadzu QP-1000 mass spectrometer combined with a GC under conditions as follows. column, 0 20 mm × 50 m glass capillary column coated with OV-101 (thickness 0.25 μm) and 3 mm × 2 m glass column packed with 2% silicon OV-17 on Cromosorb W (80—100 mesh), injector temp. 260°; column temp. programming at 2°/min from 40 to 250°; carrier gas He, split ratio 50 1 MS spectra were obtained with an ionization energy of 70 eV at 250° of the ion source temp. HPLC analyses were performed on a Radial Pak B5 (110 × 5 mm i.d) for coumarins with hexane–EtOAc (9 1) and a Radial Pak C18 (110 × 5 mm i.d) for flavanes with H_2O–MeOH (1 1) The flow rate was 1 0 ml/min and the elution of compounds was monitored at 254 and 275 nm for coumarins and flavanes, respectively.

Plant material The fresh leaves of jyabon and the 10 selected citrus were collected at a farmhouse in Akitsu-cho, Toyota-gun, Hiroshima-prefecture, Japan and at a farm of Fruit Tree Experiment Station of Hiroshima Prefecture, respectively, in May, July, September and November The fruits of jyabon and the 10 varieties of citrus were collected at the farm of the same Station as above in November. The flowers of jyabon were collected at a farm in Toyo-cho, Toyota-gun, Hiroshima-prefecture in June

Insect material Adults of *Oxycetonia jucunda* were collected at a farm of the same Station as above in May and were bred until the attraction test The wintered adults of *Unaspis yanonensis* were collected at a farm of the Akitsu Branch of National Fruit Tree Research Station in June and were bred on *Citrus unshiu* until the growth inhibition test

The essential oil constituents of the peels and fruit juice Twelve fruits of jyabon were separated into the peels and the sarcocarps. The peels (188 0 g) were ground after freezing with liquid N_2. The ground peel tissues were subjected to steam distillation The steam distillate, after saturation with NaCl, was extracted with Et_2O × 5 The unified Et_2O soln, after drying over Na_2SO_4, was evapd to give an essential oil (2 70 g) The sarcocarps were cold-pressed to give a fruit juice (590 g) The juice was steam-distilled to give 100 ml of the distillate, which was neutralized with 0 1 M NaOH and then extracted with Et_2O. The Et_2O soln, after drying, was evapd to give 68 mg of an essential oil Compositions of the essential oils obtained from the peels and fruit juice are given in Table 1 The essential oils were also obtained from the peels of the 10 selected citrus in the same manner as above. Constituents of these essential oils were characterized by GC and GC/MS

Fragrant constituents characteristic of the peels of jyabon The essential oil (1 0 g) from the peels of jyabon was chromatographed on a silica gel column with hexane, followed by

Table 4 Composition (%) of the hexane-soluble constituents from the flowers of jyabon and their organs

Compounds	Flowers	Petals	Pistils	Stamens	Sepals	Ref*
Monoterpenes†‡	52 6	75 9	68 9	15 1	55 0	
Hydrocarbons	50 7	73 3	66 9	12 1	52 0	
α-Thujene	tr	tr			tr	
α-Pinene	0 5	1 0	0 1	0 1	0 8	
Camphene	1 9	4 0	0 6	0 5	2 6	
β-Pinene	7 7	13 8	2 5	1 6	12 8	
β-Myrcene	17 8	25 0	27 0	5 1	15 7	
p-Cymene	0 1	0 1			0 3	
Limonene	9 5	11 4	17 6	2 2	6 7	
α-Phellandrene	13 0	17 7	19 0	2 6	12 6	[1]
γ-Terpinene	0 2	0 2	0 1	-	0 5	[1]
α,p-Dimethylstyrene	tr	tr	tr			[1]
Terpinolene	tr	0 1				[1]
Oxygenated compds	1 6	2 6	1 7	0 5	0 8	
Linalool	1 2	1 9	1 6	0 5	0 7	
Citronellal	tr	0 1			0 1	
Citronellol	tr	0 1	0 1		tr	
Nerol	tr	0 1	tr			
Neral	tr	tr				
Geraniol	tr	0 1	tr		tr	
Geranial	0 1	0 2		tr		
Neryl acetate	tr	0 1				
Sesquiterpenes†‡	7 7	10 3	8 1	2 9	10 4	
α-Copaene	0 1	0 1	tr	tr	0 1	[2]
β-Elemene	0 1	0 1	tr	tr	tr	[2]
Caryophyllene	4 1	5 1	4 4	1 4	5 7	
β-Bisabolene	2 2	3 2	1 6	0 6	3 2	[2]
α-Humulene	0 3	0 3	0 2	0 1	0 4	[2]
allo-Aromadendrene	0 2	0 4	0 2	0 1	0 3	[2]
α-Elemene	0 4	0 7	0 1	0 1	0 6	[2]
δ-Cadinene	0 2	0 3	0 1	0 1	0 3	[2]
Others†	38 1	13 8	23 0	82 0	34 6	
Fatty acid methyl esters	37 2	13 6	21 3	78 5	33 3	

* References for identification of the constituents
† The figures include percentages of the other unidentified compounds
‡ Described in the order of elution from an OV-101 GC column
tr trace (<0 1%)

hexane–EtOAc mixtures with an increasing EtOAc content, to give two fractions (A) and (B) The fractions A (173 mg) and B (73 mg) were eluted with hexane–EtOAc (9 1) The fraction A was composed of 1,8-cineol (20 2%), citronellal (12 9%), neral (11 6%), *cis*-carveol (11 1%), anthemol (9 1%), 1-*p*-menthen-9-al (12 3%) and unidentified constituents (total 22 8%) The hexane soln of the fraction A (*ca* 70 mg) was poured into a sample bottle The soln in this bottle was allowed to stand for 10 hr at room temp after removing the cap The remaining soln was then subjected to GC analysis to give citronellal (16 5%), neral (14 9%), *cis*-carveol (14 3%), anthemol (11 1%), 1-*p*-menthen-9-al (16 3%) and unidentified constituents (total 26.9%) When 1,8-cineol (*ca* 10 mg) dissolved in hexane (0 1 ml) was added to the soln, the resulting soln reproduced the fragrance characteristic of jyabon This soln was, on GC analysis, composed of 1,8-cineol (25 1%), citronellal (13 9%), neral (15 6%), *cis*-carveol (14 1%), anthemol (8 9%), 1-*p*-menthen-9-al (12 1%) and unidentified constituents (total 10 3%)

Isolation and identification of aurapten (**1**), *psoralen* (**2**), *hesperidin* (**3**) *and naringin* (**4**) The peels (269 0 g) of the fruits of jyabon were immersed in MeOH for three months at room temp in N_2 The MeOH soln was partitioned between hexane and MeOH to give a hexane-soluble fraction (2 5 g) This fraction was then chromatographed on silica gel with hexane–EtOAc (4 · 1) to give aurapten (**1**) (56 mg, mp 66 0–66 5°) The residual MeOH soln was evapd to a small vol under red pressure and the concentrate dissolved in H_2O The aq soln was successively extracted with Et_2O, EtOAc and BuOH An Et_2O soln (0 3 g) was, after removal of the solvent, chromatographed on a silica gel column with hexane, followed by hexane–EtOAc mixtures with an increasing EtOAc content A fraction (48 mg) eluted with hexane–EtOAc (7 3) was re-chromatographed on a silica gel column with the same solvent to give psoralen (**2**) (45 mg, mp 159–160°) An EtOAc soln (0 7 g) was, after removal of the solvent, subjected to prep TLC with $CHCl_3$–MeOH–H_2O (16 6 1) to give hesperidin (**3**) (48 mg, mp 240–241°) and naringin

Table 5 Attraction rates* of hexane extracts from the whole flowers of jyabon, *C unshiu* Marc. and their organs and the rates of major constituents in the hexane extract from the petals of jyabon for *O jucunda*

	Concentration/ppm		
	1	0.1	0.01
Jyabon			
Whole flowers	58.7	20.1	—†
Petals	79.2	25.2	—
Pistils	87.9	30.2	—
Stamens	22.5	7.1	—
Sepals	47.8	16.8	—
C unshiu Marc.			
Whole flowers	6.1	—	—
Petals	9.2	—	—
Pistils	5.1	—	—
Stamens	3.1	—	—
Sepals	4.1	—	—
Major constituents in the petals of jyabon			
β-Pinene	2.9	16.5	0
β-Myrcene	11.4	76.4	10.1
Limonene	0	3.7	0
α-Phellandrene	4.8	13.1	0

*Attraction rate was determined by the following equation.

Attraction rate (%) = [(P − Q/P + Q)] × 100

Where P is the number of the chafers on the sample side and Q on the control side

† Not measured.

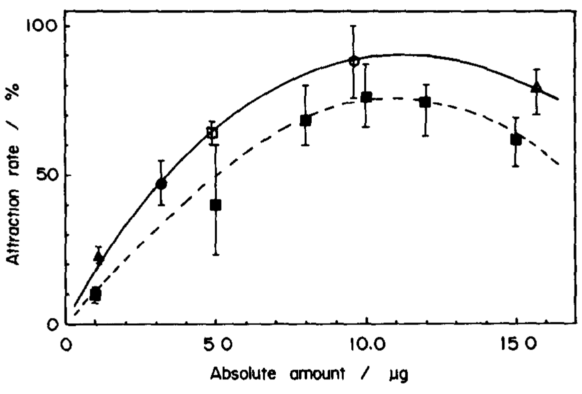

Fig. 2. Relationship between the absolute amounts (determined on the basis of the standard curve on GC) of β-myrcene present in the solns (1.0 ppm) of the hexane extracts from the whole flowers (□), petals (△), pistils (○), stamens (▲) and sepals (●) of jyabon and the attraction rates of the hexane extracts and relationship between the absolute amounts of authentic β-myrcene (■) and their attraction rates.

Table 6 Composition (%) of the main constituents of the essential oils from the new and old leaves of jyabon collected in May

Compounds	New leaves	Old leaves	Refs*
Monoterpenoids†‡	79.7	64.0	
Hydrocarbons	62.7	56.5	
β-Pinene	18.8	19.3	
β-Myrcene	11.1	12.3	[1]
α-Phellandrene	12.0	12.0	[1]
Oxygenated compds	17.0	7.5	
Linalool	8.1	0.7	
Citronellal	1.0	3.2	[1]
Citronellol	0.9	—	
Nerol	0.4	2.9	
Neral	2.7	—	
Geranial	3.5	—	
Sesquiterpenoids†‡	15.6	31.3	
Caryophyllene	8.3	15.0	
β-Bisabolene	2.3	5.5	[2]
α-Elemene	2.8	7.0	[2]
Others	4.7	4.7	

* References were identification of the constituents

† The figures include percentages of the other unidentified compounds

‡ Described in the order of elution from an OV-101 GC column

Table 7 Correlation coefficients of jyabon to 10 selected citrus on the basis of the composition of the essential oil constituents from the new and old leaves collected in May

Citrus	New leaves	Old leaves
Poncirus trifoliata Rafin	0.24	—*
Citrus limon Swingle	0.31	0.11
C. aurantifolia	−0.09	−0.07
C grandis var anseikan Hort. ex Tanaka	0.86	0.66
C natsudaidai Hayata	0.09	0.28
C junos Sieb ex Tanaka	0.02	0.02
C sphaerocarpa Hort · ex Tanaka	0.35	0.10
C sudachi Hort. ex Shirai	0.01	0.01
C. unshiu Marc.	0.15	0.08
Fortunella japonica Swingle	—†	−0.12

* The leaves could not be collected, because *P trifoliata* is deciduous

† The leaves could not be collected, because they were not out in May.

(4) (140 mg, mp 84.0–84.5°) A BuOH soln (2.4 g) was, after removal of the solvent, subjected to prep. TLC with $CHCl_3$–MeOH–H_2O (16.6.1) to give **3** (107 mg) and **4** (142 mg). **1–4** were identified by comparison of their physical data and IR, NMR and MS data with those described, respectively, in refs [8–10] and [11, 12].

Following the procedure similar to that for the peels of jyabon, the peels of the 10 selected citrus were separately treated to give the hexane, Et_2O, EtOAc and BuOH extracts. Each of the extracts was subjected to HPLC analysis to identify **1–4**.

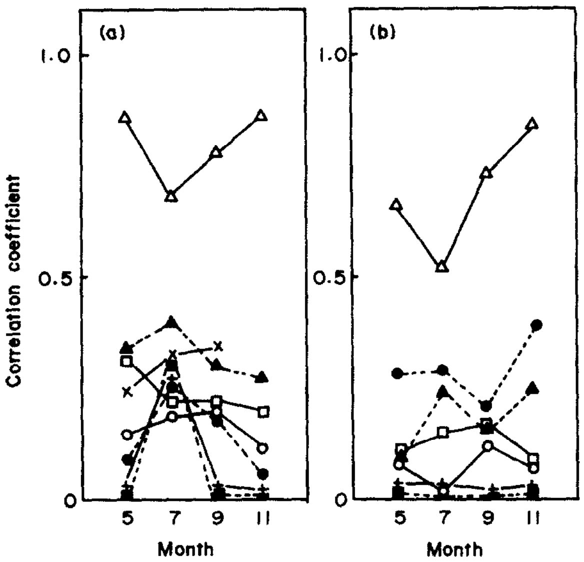

Fig 3 Seasonal variations in the correlation coefficients of jyabon to 10 selected citrus on the basis of the compositions of essential oil constituents from the new (a) and old (b) leaves *Poncirus trifoliata* Rafin (×), *Citrus limon* Burm f (□), *C grandis* var anseikan Hort ex Tanaka (△), *C natsudaidai* Hayata (●), *C. junos* Sieb ex Tanaka (+), *C sphaerocarpa* Hort ex Tanaka (▲), *C. sudachi* Hort ex Shirai (■) and *C unshiu* Marc (○) Correlation coefficients of jyabon to *C aurantifolia* Swingle and *Fortunella japonica* Swingle were too low (< 0 01) to plot in these figures

Growth inhibition test of the essential oil, coumarins and flavanes for the arrowhead scale insect. Agar (0 25 g) was added to 49 ml of the aq soln of crystalviolet (20 ppm) and streptomycin sulphate (10 ppm) and the soln was heated at 120° in an autoclave. To the hot soln, 1 ml of the sample soln (5 mg/ml) in Me_2CO or H_2O was added Before coagulation of the agar soln. the segment of the leaf of *C unshiu* was placed on this agar soln The segment (3 0–4 0 cm in width and 4.0–6 0 cm in length) was made by crosscut at each site of 10 mm from a leaf-stalk and a pointed head of a leaf Incorporation of the sample into the segment of the leaf was *ca* 10 ng/g segment for psoralen (**2**) by HPLC analysis Then, larvae of the arrowhead scale insect were placed on this segment, and the body length and body width of scale larvae on the segment were measured (Fig 1) twice a week for a month After the breeding on the segment of the leaf for a month, number of deaths and survivors of the first stage and the second stage insects were counted under a microscope Further females and males of the second stage insects were distinguished in the similar manner The results are given in Table 2

Attractants for the citrus flower chafer The fresh flowers (380 g) of jyabon were ground after freezing with liquid N_2 The ground flower-tissues were immersed in MeOH (1 l) for 2 weeks at room temp in N_2 The MeOH soln, after filtration, was partitioned between hexane and MeOH Removal of the solvent from the hexane soln, after drying over dry $MgSO_4$, gave a hexane extract (3 7 g) On the other hand, the flowers (588 1 g) of jyabon were separated into petals (214 5 g), pistils (80 2 g), stamens (166 4 g) and sepals (125 0 g) A hexane extract was obtained from each of these separated organs in the manner similar to that in the case of the whole flower of jyabon The hexane extract gave 2 47 g of an essential oil for the petals, 0 64 g for the pistils, 1 56 g for the stamens and 1 50 g for the sepals Compositions of the essential oil constituents in the whole flowers of jyabon and their organs are given in Table 4

A hexane extract was also obtained from the whole flowers of *C unshiu* and their organs in the manner similar to that in the case of the whole flowers of jyabon The essential oils from the whole flowers of *C unshiu* and their organs were mainly composed of γ-terpinene (16 0–36 2%), limonene (3 0–10 9%) and β-elemene (5 1–9 0%)

Attraction test of the constituents for the citrus flower chafer All solns except for authentic materials were prepared as 0 001% Triton X-100 aq solns in concns of 0 1 and 1 0 ppm The solns of the authentic materials were prepared in concns of 1 0, 0 1 and 0 01 ppm The authentic materials used were β-pinene, 99% pure on GC, n_D^{25} 1 4764, $[\alpha]_D^{25}$ −22 6° (neat), β-myrcene, 99% pure on GC, n_D^{25} 1 4693, limonene, 98% pure on GC, n_D^{25} 1 4703, $[\alpha]_D^{25}$ +123 1 (neat) and α-phellandrene, 99% pure on GC, n_D^{25} 1 4730, $[\alpha]_D^{25}$ −94 30° (neat) As a control, 0 001% Triton X-100 aq soln was used for all the tests Odd numbered citrus flower chafers (more than 11 chafers) were placed on a centre of the olfactometer [5] After airing for 5 min, the number of the chafers moved to the sample side and the control side were counted The attraction test was performed × 3 and the attraction rate was obtained as an average of three runs (Table 5)

The essential oil constituents of the leaves. Following the method in the case of the peels, the fresh leaves (99 0 g for the new leaves in May and 126 0 g for the old leaves in May) of jyabon were steam-distilled to give an essential oil (426 mg for the new leaves and 386 mg for the old leaves) The essential oils were also obtained from the new and old leaves of the 10 selected citrus in the same manner as above Constituents of these essential oils were characterized by GC and GC/MS (Table 6)

Calculation of correlation coefficients According to Hanshu's theory [7], correlation coefficients were calculated by use of the program [13]

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