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SESQUITERPENE LACTONES FROM PYRETHRUM FLOWERS

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Key Word Index—*Chrysanthemum cinerariaefolium*, Compositae, pyrethrum flowers, sesquiterpene lactones, germacranolides, tatridins, ^{13}C NMR, flavonoids, growth inhibitor

Abstract—Three new sesquiterpene lactones of the germacranolide-type [(11*R*)-11,13-dihydrotatridin-A, (11*R*)-11,13-dihydrotatridin-B and (11*R*)-6-*O*-β-D-glucosyl-11,13-dihydrotatridin-B] were isolated from the flower heads of *Chrysanthemum cinerariaefolium*, together with the known sesquiterpene lactones tatridin-A, tatridin-B and dihydro-β-cyclopyrethrosin and the known flavonoids jaceidin, apigenin, luteolin, apigenin-7-galacturonic acid methyl ester and apigenin-7-glucuronic acid All of the compounds isolated inhibited root growth of Chinese cabbage seedlings.

INTRODUCTION

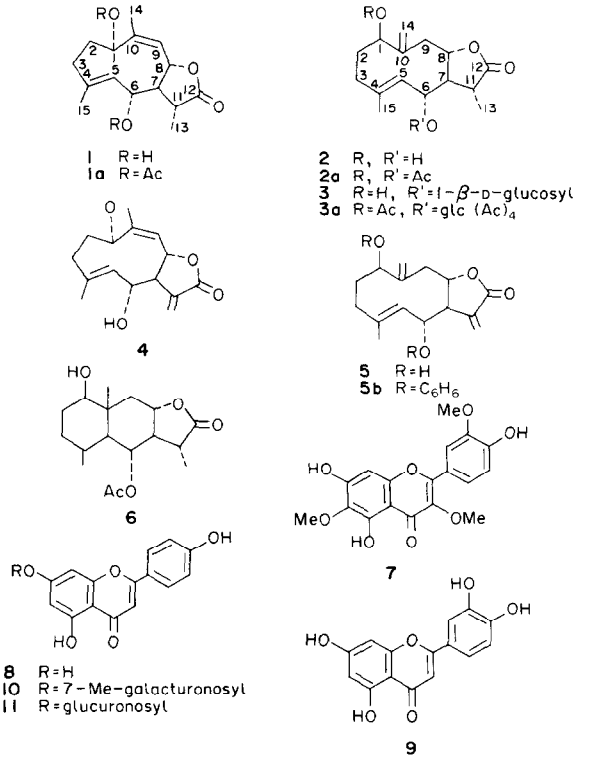
Besides the well-known insecticidal ingredients, several flavonoids and sesquiterpene lactones have been isolated from *Chrysanthemum cinerariaefolium* Vis. [1–4] In our previous paper [5], we reported that an extract from the flower heads of *C. cinerariaefolium* inhibited the growth of Chinese cabbage roots We now report the isolation from the flower heads of three new sesquiterpene lactones, together with three other sesquiterpene lactones and five flavonoids They all had significant plant growth inhibiting activity.

RESULTS AND DISCUSSION

Flower heads of *C. cinerariaefolium* were extracted with diethyl ether, hot methanol and boiling water, successively. The methanol extract, which had the strongest inhibiting effect on the growth of Chinese cabbage seedlings, was repeatedly separated by Si gel chromatography to give 11 compounds (**1**–**11**).

Compound **1**, $C_{15}H_{22}O_4$, had $[\alpha]_D^{21} - 27°$ (MeOH). IR absorption bands at 3400, 1770, 1660 and 1600 cm^{-1} indicated the presence of a hydroxyl group(s), a γ-lactone and a double bond(s) The ^1H NMR spectrum of **1** revealed two olefinic methyl groups(C-14 and C-15), δ 1.76 (6H, *br s*); a secondary methyl group (C-13), δ 1.32 (3H, *d*, $J_{11,13} = 7$ Hz); and two olefinic protons H-5 and H-9, δ 4.93 (1H, *br d*, $J_{5,6} = 10.5$ Hz) and 5.19 (1H, *br d*, $J_{8,9} = 10\,5$ Hz). A broadened doublet of doublets ($J_{1,2} = 3$, 10.5 Hz) at δ 4.40 and a broadened triplet ($J_{5,6} = 10.5$, $J_{6,7} = 10.5$ Hz) at δ 4.46 were assigned to CHOH at C-1 and C-6, respectively. The proton under the lactone closed to C-8 appeared as a triplet ($J_{7,8} = 10.5$, $J_{8,9} = 10\,5$ Hz) at δ 4.67. A doublet of quartets ($J_{7,11} = 10.5$, $J_{11,13} = 7$ Hz) at δ 2.74 was assigned to H-11 which was presumed to be pseudo-axial because of the value of $J_{7,11}$. Acetylation of **1** gave a crystalline diacetate, **1a**, mp 165–166°. In the ^1H NMR spectrum of **1a**, two 3H singlets for the acetyl methyl groups appeared at δ 2.00 and 2.03 and the signals of H-1 and H-6 moved downfield to δ 5.40 and 5 45, respectively.

The data for **1** were similar to those of tatridin-A (**4**)



except for the presence of a secondary methyl group(C-13). Therefore, **1** was assumed to be (11*R*)-11,13-dihydrotatridin-A. The structure was finally established by direct comparison with a sample derived from authentic **4** by sodium borohydride reduction

Compound **2** was obtained as colourless needles, mp 166–168°, $[\alpha]_D^{21} + 43°$. The mass spectrum exhibited a molecular ion at *m/z* 266 and peaks at *m/z* 248 and 230 for the stepwise elimination of the two molecules of water. Its IR spectrum showed peaks for a hydroxyl group(s), a γ-lactone and a double bond(s). The ^1H NMR spectrum of **2** exhibited a doublet at δ 1.34 (3H, $J_{11,13} = 7$ Hz), which

was assigned to a secondary methyl group on the lactone ring, a signal at δ 1.70 (3H) was attributed to an olefinic methyl group (C-15), and a broadened doublet ($J_{5,6}$ = 10 Hz) at δ 5.30 was assigned to H-5 A broadened signal appeared at δ 4 00 (2H) for H-1 and H-8, and the C-1 and C-6 hydroxyl protons gave two broadened signals at δ 2 62 and 2 85 Two protons of the methylene group (C-14) appeared as two broadened signals at δ 5.01 and 5 08 H-6 appeared as a doublet of triplets ($J_{6,OH}$ = 4 5, $J_{5,6}$ = 10, $J_{6,7}$ = 10 Hz) at δ 4 40 A doublet of quartets ($J_{7,11}$ = 10, $J_{11,13}$ = 7 Hz) at δ 2 60 was attributed to H-11 In the ^{1}H NMR spectrum of the acetate of **2** (**2a**), two three-proton signals for the acetyl methyl groups, instead of the two hydroxyl groups, appeared at δ 2 05 The H-1 and H-6 signals moved downfield to δ 5 23 (*br*) and 5 42 (*t*, $J_{5,6}$ = 10, $J_{6,7}$ = 10 Hz), respectively, and both the protons at C-14 at δ 5 01 and 5 08 moved to δ 5 24. H-8 clearly appeared as a doublet of triplets ($J_{8,9}$ = 3, $J_{7,8}$ = 10, $J_{8,9}$ = 10 Hz) at δ 4 09.

Based on these data and direct comparison with a sample derived from authentic tatridin-B (**5**), **2** was identified as (11*R*)-11,13-dihydrotatridin-B

Compound **3**, $C_{21}H_{32}O_9$, was an amorphous powder The IR spectrum showed peaks for hydroxyl groups, a γ-lactone and a double bond(s) The ^{1}H NMR spectrum of **3** was quite similar to that of **2** except for the additional presence of the sugar moiety Acetylation of **3** gave the penta-acetate, **3a** In the ^{1}H NMR spectrum, the H-1 signal at δ 4 20 moved downfield to δ 5.10 and the protons at C-14 moved to δ 5 10 and 5 20 Compound **3** on hydrolysis with β-glucosidase, yielded D-glucose and **2** Therefore, the structure of **3** was (11*R*)-6-*O*-β-D-glucosyl-11,13-dihydrotatridin-B Moreover, the ^{13}C NMR data (Table 1) supported this structure.

Compounds **1**–**3** have not been reported as naturally occurring sesquiterpene lactones

Compounds **4** and **5** are tatridin-A [6–8] and tatridin-B [6, 9], respectively, the identities of **4** and the dibenzoate (**5b**) of **5** were confirmed by direct comparison (mmp and ^{1}H NMR and mass spectra) with authentic samples

Compounds **6**–**11** were identified as dihydro-β-cyclopyrethrosin, jaceidin, apigenin, luteolin, apigenin-7-galacturonic acid methyl ester and apigenin-7-glucuronic acid, respectively

Compounds **4** and **5** and **7**–**10** have never been isolated from this plant

Table 1 ^{13}C NMR spectral data of **3** (CD_3OD, TMS as int standard)

C No	δ	C No.	δ
1	72 6 *d*	12	181 5 *s*
2	32 1 *t*	13	18 2 *q*
3	34 5 *t*	14	114 5 *t*
4	140 9 *s*	15	17 2 *q*
5	128 0 *d*	G1	99 7 *d*
6	76 9 *d*	G2	74 8 *d*
7	42 0 *d*	G3 or G5	77 8 *d*
8	81 9 *d*	G4	71.7 *d*
9	42 5 *t*	G5 or G3	78 3 *d*
10	148 0 *s*	G6	62 8 *t*
11	56 9 *d*		

Physiological activity

The plant growth inhibiting activity of these compounds was investigated with Chinese cabbage seedlings (Table 2). All compounds inhibited root growth Compounds **7**–**9** inhibited root growth at 5 ppm, whereas **3**, **10** and **11** showed relatively mild effects However, these compounds scarcely inhibited the germination of Chinese cabbage seeds and almost all the seeds germinated within 24 hr.

Thus, all 11 compounds may be said to contribute to the growth inhibiting activity in the flower heads of *C cinerariaefolium*

It has been reported that sesquiterpenes of the α-methylene γ-lactone type, such as heliangin and pyrethrosin, inhibited the elongation of the coleoptile section of *Avena* [10–12] However, in our investigation, **1**–**3** and **6**, which are of the dihydro type as well as **4** and **5** with an α-methylene on the γ-lactone ring system, inhibited the growth of Chinese cabbage roots

Table 2 Effect of isolated compounds on growth of roots of Chinese cabbage seedlings

	Concentration (ppm)				
Compound	1	5	10	50	100
1	91	89	75*	—	
2	94	89	71*	—	—
3	—	—	86	84	64*
4	97	86	78*	—	—
5	97	85	75*	—	—
6	103	112	79*	—	—
7	104	77*	—	—	
8	99	76*	74*	—	—
9	100	72*	71*	—	—
10	—	—	102	100	77*
11	—	—	100	89	70*

Each value represents the mean of the root lengths as a percentage of the control (41 ± 7 7 mm)

*Significantly different from control at the 5% level, *t*-test

EXPERIMENTAL

Mps are uncorr ^{1}H NMR 100 MHz, TMS as int standard GC OV-101, 100–160

Extraction and separation The flower heads (1 2 kg) of *C cinerariaefolium* Vis, purchased in Tokyo, were extracted with Et_2O (4 l), hot MeOH (4 l) and boiling H_2O (4 l), successively The MeOH extract (95 g) was chromatographed over Si gel with mixtures of $CHCl_3$–MeOH The $CHCl_3$–MeOH (25 1) fraction was rechromatographed over Si gel to give **6** (110 mg) and **7** (20 mg) The $CHCl_3$–MeOH (15 1) fraction was subjected to CC on Si gel followed by prep TLC (Si gel) with C_6H_6–Me_2CO (4 1, ×2) to give **1** (25 mg), **2** (12 mg), **4** (7 mg) and **5** (7 mg) The $CHCl_3$–MeOH (10 1) fraction gave **8** (110 mg) and **9** (50 mg) The $CHCl_3$–MeOH (2 1) fraction, after addition of H_2O, was separated into filtrate and residue The filtrate was chromatographed on charcoal with MeOH as eluate to give **3** (70 mg) CC of the residue over Si gel eluate with mixtures of $CHCl_3$–MeOH gave **10** (130 mg) and **11** (70 mg) in the $CHCl_3$–MeOH (5 1) fraction

Bioassay Aq solns of **3**, **10** and **11** were prepared at concns of 10, 50 and 100 ppm and those of **1**, **2** and **4**–**9**, which were all sparingly soluble in H_2O, at 1, 5 and 10 ppm (or 1 and 5 ppm). H_2O was used as control soln

A piece of filter paper, impregnated with 7 ml test soln, was placed in a Petri dish (9 × 1 5 cm) 20 seeds of Chinese cabbage (*Brassica rapa* L var *pervidis* Bailey) were placed on the filter paper and kept at 25° in the dark After 3 days, the lengths of the roots were measured The expt was replicated × 3

(11*R*)-11,13-*Dihydrotatridin-A* (**1**) Amorphous powder, $[\alpha]_D^{21} - 29°$ (MeOH, *c* 1 5) MS *m/z* 266 $[M]^+$, 248, 193, 175, 137, 123, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3400, 1700, 1660, 1600, 1130 Compound **1** on acetylation with Ac_2O–pyridine at room temp gave **1a** (diacetate of **1**), colourless needles, mp 165–166° (EtOH) ^{1}H NMR ($CDCl_3$) δ 1 30 (3H, *d*, *J* = 7 Hz, H-13), 1 80 (3H, *d*, *J* = 1 Hz, H-14), 1 93 (3H, *br s*, H-15), 2 00 and 2 03 (each 3H, *s*, Ac × 3), 2 58 (1H, *dq*, *J* = 10, 7 Hz, H-11), 4 79 (1H, *t*, *J* = 10 Hz, H-8), 4 90 (1H, *br d*, *J* = 10 Hz, H-5), 5 30 (1H, *br d*, *J* = 10 Hz, H-9), 5 40 (1H, *mix*, H-1), 5 45 (1H, *t*, *J* = 10 Hz, H-6) The mp of **1a** was not depressed on admixture with the synthetic sample.

Synthesis of **1a** Tatridin-A (10 mg) in EtOH (1 ml) was treated with $NaBH_4$ (2 mg) for 30 min with stirring After acidification to pH 6 with 10% HOAc, the EtOH was removed by evaporation The residue was taken up in $CHCl_3$ and the extract dried and acetylated with Ac_2O–pyridine to give **1a** (7 mg), mp 165–166°

(11*R*)-11,13-*Dihydrotatridin-B* (**2**) Colourless needles, mp 167–168° (EtOH), $[\alpha]_D^{21} + 43°$ (MeOH, *c* 0 65), MS *m/z* 266 $[M]^+$, 248, 230, 220, 202, 193, 175, 157, IR ν_{max}^{KBr} cm^{-1} 3300, 2920, 1760, 1640, 1290 The mp was not depressed on admixture with the synthetic sample Compound **2** on acetylation with Ac_2O–pyridine at room temp gave **2a** (diacetate of **2**) as an amorphous powder ^{1}H NMR ($CDCl_3$) δ 1 35 (3H, *d*, *J* = 7 Hz, H-13), 1 85 (3H, *s*, H-15), 2 05 (6H, *s*, Ac × 2), 2 29 (1H, *q*, *J* = 10 Hz, H-7), 2 59 (1H, *dq*, *J* = 10, 7 Hz, H-11), 4 09 (1H, *dt*, *J* = 3, 10 Hz, H-8), 5 02 (1H, *br d*, *J* = 10 Hz, H-5), 5 23 (1H, *mix*, H-1), 5 24 (2H, *br*, H-14), 5 42 (1H, *t*, *J* = 10 Hz, H-6)

Synthesis of **2** Tatridin-B (10 mg) was acetylated by the same procedure as that used for the synthesis of **1a** After removal of the EtOH, the residue was extracted with EtOAc, and the extract subjected to prep TLC with C_6H_6–Me_2CO (2 1) to give **2** (4 mg), mp 165–167°

(11*R*)-6-O-β-D-*Glucosyl*-11,13-*dihydrotatridin-B* (**3**) Amorphous powder, $[\alpha]_D^{25} - 25°$ (MeOH, *c* 1 0), CIMS *m/z* 429 $[MH]^+$, 267, 250, 248, 231, 203, 145; IR ν_{max}^{KBr} cm^{-1} 3350, 2920, 1750, 1640, 1080, ^{1}H NMR (pyridine) δ 1 43 (3H, *d*, *J* = 7 Hz, H-13), 1 80 (3H, *br s*, H-15), 2 75 (1H, *dq*, *J* = 10, 7 Hz, H-11), 4 20 (2H, *mix*, H-1, H-8), 4 50 (1H, *t*, *J* = 10 Hz, H-6), 5 00 (2H, *br*, H-14), 5 30 (1H, *br d*, *J* = 10 Hz, H-5), ^{13}C NMR Table 1 Compound **3** on acetylation with Ac_2O–pyridine gave **3a** (penta-acetate of **3**) as an amorphous powder. CIMS *m/z* 639 $[MH]^+$, 331, 291, 259, 231, 169; ^{1}H NMR ($CDCl_3$) δ 1 30 (3H, *d*, *J* = 7 Hz, H-13), 1 74 (3H, *s*, H-15), 2 00–2 10 (15H, Ac × 5), 2 30 (1H, *m*, H-7), 2 60 (1H, *mix*, H-11), 4 00 (1H, *mix*, H-8), 4 44 (1H, *t*, *J* = 10 Hz, H-6), 5.10 (1H, *mix*, H-1), 5 10 and 5 20 (each 1H, *br*, H-14), 5 15 (1H, *br d*, *J* = 10 Hz, H-5)

To the aq. acetate buffer soln (pH 5) of **3**, β-glucosidase was added, and the mixture kept at 37° for 3 hr The hydrolysate was treated with EtOAc and the extract separated by prep TLC with C_6H_6–Me_2CO (3.1) to give the aglycone (11*R*)-11,13-dihydrotatridin-B (**2**), mp 165–167° The mp was not depressed on admixture with **2** The residue gave D-glucose as the sugar (GC)

Tatridin-A (**4**) Colourless needles, mp 174–176° (MeOH) MS *m/z*. 264 $[M]^+$, 246, 180, 149, 121, IR ν_{max}^{KBr} cm^{-1}. 3300, 2920, 1760, 1650, 1270, 1150, 1000, 960 These data and ^{1}H NMR data agreed closely with the lit [7] The mp was not depressed on admixture with an authentic sample

Tatridin-B (**5**) Compound **5**, obtained as an amorphous powder, could not be crystallized and was converted to the dibenzoate, **5b**, in the usual manner; colourless needles, mp 217–218° (EtOH) MS *m/z*. 472 $[M]^+$, 315, 228, 105, IR ν_{max}^{KBr} cm^{-1} 2950, 1765, 1705, 1270, 1100 ^{1}H NMR data for **5b** were in good agreement with the lit [6] The mp of **5b** was not depressed on admixture with an authentic sample.

Dihydro-β-cyclopyrethrosin (**6**) Colourless needles, mp 213–214° (EtOH–isopropyl ether) $[\alpha]_D^{21} + 69°$ (MeOH, *c*, 0 5), MS *m/z*. 248 $[M - CH_2C{=}O, H_2O]^+$ These data and the IR and ^{1}H NMR data agreed with the lit. [4]

Jaceidin (**7**). Yellow powder, mp 97–99° ($CHCl_3$). MS *m/z* 360 $[M]^+$, UV λ_{max}^{MeOH} nm· 256, 272, 354; $\lambda_{max}^{AlCl_3}$ nm 271, 284, 301, 385, λ_{max}^{NaOAc} nm 273, 317, 394 These data and the IR and ^{1}H NMR data agreed with the lit [13]

Apigenin (**8**) Yellow powder, mp 340° (MeOH–H_2O) MS *m/z* 270 $[M]^+$, UV λ_{max}^{MeOH} nm: 268, 334, $\lambda_{max}^{AlCl_3}$ nm. 276, 302, 348, λ_{max}^{NaOAc} nm 270, 300, 344 The IR and ^{1}H NMR data were identical to the values of authentic sample and the mp was not depressed on admixture with the authentic sample

Luteolin (**9**) Yellow needles, dec. 318° (MeOH–H_2O) MS *m/z*. 286 $[M]^+$; UV λ_{max}^{MeOH} nm 230, 265, 294, $\lambda_{max}^{AlCl_3}$ nm. 267, λ_{max}^{NaOAc} nm· 273, 303, 330 The IR and ^{1}H NMR data agreed with the values of an authentic sample

Apigenin-7-galacturonic acid methyl ester (**10**) Pale yellow needles, mp 242–243° (MeOH–H_2O). MS *m/z* 460 $[M]^+$, UV λ_{max}^{MeOH} nm 268, 335, $\lambda_{max}^{AlCl_3}$ nm 276, 299, 347, λ_{max}^{NaOAc} nm 268, 345, 390. These data and the IR and ^{1}H NMR data agreed with the lit [14]

When **10** (10 mg) and 20% H_2SO_4 (5 ml) were allowed to stand at 100° for 1 hr the reaction mixture gave galacturonic acid (GC) and apigenin as aglycone (mmp, TLC)

Apigenin-7-glucuronic acid (**11**) Yellow powder, mp 176–178° (MeOH–H_2O) MS *m/z* 270 $[M - C_6H_8O_6]^+$, UV λ_{max}^{MeOH} nm 269, 335, $\lambda_{max}^{AlCl_3}$ nm 275, 299, 348, λ_{max}^{NaOAc} nm 269, 347, 390 These data and the IR and ^{1}H NMR data agreed with the lit [13]

When **11** was heated with 20% H_2SO_4 at 100° for 1 hr, it gave glucuronic acid (GC) and apigenin (mmp, TLC)

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