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PLANT GROWTH RETARDANT ACTIVITY OF 4-HOMOISOTWISTANE DERIVATIVES*

Koji Aigami, Yoshiaki Inamoto, Naotake Takaishi and Yoshiaki Fujikura
Industrial Research Laboratories, Kao Soap Company, Ltd. 1334 Minatoyakushubata, Wakayama 640–91, Japan

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Abstract—A variety of simple derivatives of 3-substituted 4-homoisotwistane derivatives were prepared, and their effect on the growth of cucumber seedlings in complete darkness was investigated. The 3-hydroxy derivative was found to show a strong inhibitory activity at 50 μg/ml, so a series of other hydroxy derivatives of 4-homoisotwistane, endo-2-, exo-2-, and 5-hydroxy- and exo-2,3-dihydroxy-4-homoisotwistane were prepared in order to obtain information on structure–activity relationships. The endo-2-hydroxy derivative inhibited the growth of cucumber and the germination of lettuce seed at 12.5 μg/ml. All the hydroxy derivatives tested increased the number of adventitious roots in hypocotyls of kidney bean at 100 μg/ml, but they inhibited root formation at the lowest part of the cuttings, and the effect was again exhibited most strongly by the endo-2-hydroxy compound. It is suggested that the 2- and 3-hydroxy derivatives possess a potent activity as plant growth retardants.

INTRODUCTION

In the previous paper of this series [1], the antiviral activity of some derivatives [2] of 4-homoisotwistane-(tricyclo[$5.3.1.0^{3,8}$]undecane, **3**) [3] was reported. The parent hydrocarbon **3** is a key intermediate in the adamantane rearrangement of various tricycloundecane precursors [4–6], and was demonstrated theoretically [6] and experimentally [5–7] to be one of the most stable of the tricycloundecane isomers.

Since 4-homoisotwistane (**3**) has the same skeletal structure as that of patchouli alcohol and seychellene, which are naturally occurring sesquiterpenoids and appear as constituents of some essential oils, derivatives of **3** may be expected to show some physiological activity. In this paper, the effects of **3** and its derivatives on the growth of cucumber seedlings, the germination of lettuce seeds, and adventitious root formation of kidney bean cuttings are described.

RESULTS

Effect on cucumber seedlings. The effects of a variety of 3-substituted 4-homoisotwistanes on the growth of hypocotyls of cucumber seedlings in complete darkness are summarized in Table 1. It was found that the growth of cucumber seedlings was greatly affected by the type of substituent in the 4-homoisotwistyl derivatives. For example, the 3-hydroxy derivative **7** was quite effective, while the nitrile (**20**), amides having lower *N*-alkyl moieties (**16**, **19**a), acetamide (**5**), aminomethyl derivative (**21**), and the 3-hydroxymethyl derivative (**14**) were only moderately active. Esters (**13**, **18**), amides having higher *N*-alkyl moieties (**19**b, **19**c, **19**d), the amine hydrochloride (**6**), the acid (**12**) and 4-homoisotwistane itself (**3**) were almost inactive at 100 μg/ml.

The difference in the activities of two alcohols, **7** and **14**, shows that the position and configuration of a functional group may greatly affect the activity. This led us to examine a series of hydroxy derivatives of **3**. As shown in Table 2, the endo-2- (**11**) and exo-2-hydroxy derivatives (**9**), in addition to the 3-hydroxy isomer (**7**), were found to be quite active and completely inhibited the growth of cucumber hypocotyls at 25, 100 and 100 μg/ml, respectively. However, the seedlings were not dead, but formed a callus-like tissue at the cotyledon. The 3-hydroxymethyl (**14**), exo-2,3-dihydroxy (**22**), and 5-hydroxy derivatives (**24**) showed only a moderate activity.

Table 1. Effects of 4-homoisotwistane and its 3-substituted derivatives on the growth of hypocotyls of cucumber seedlings

Compound*	Concentration (μg/ml) 500	100	Compound*	Concentration (μg/ml) 500	100
3	89	91	**18**a	69	95
5	10	32	**18**b	89	92
6	36	100	**18**c	70	92
7	0	0	**19**a	8	55
12	41	76	**19**b	10	78
13	36	83	**19**c	43	88
14	5	65	**19**d	59	90
16	13	45	**20**	15	60
			21	5	64

* Each value represents the mean of the hypocotyl lengths as a % of control.

Effect on the germination of lettuce seeds. As shown in

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* Part 3 in the series "Biologically active policycloalkanes": previous paper [1].

Table 3, the endo-2-hydroxy compound (**11**) almost completely inhibited the germination of lettuce seeds under light at 12.5 μg/ml, while the derivatives **14** and **24** showed a relatively mild effect. Activity differences between these compounds were similar to those found for the growth of cucumber hypocotyls. However, the order of the activity of the 3-hydroxy compound (**7**) and the exo-2,3-dihydroxy derivative (**22**) for this test was the opposite of that for cucumber test, **22** being more potent than **7**.

Table 2. Effects on 4-homoisotwistanols on the growth of hypocotyls of cucumber seedlings

	Concentration (μg/ml)						
Compound*	3.1	6.2	12.5	25	50	100	200
7	†	83	71	62	18	0	0
9	†	98	90	85	50	0	0
11	95	66	18	0	0	0	0
14	†	†	†	86	75	65	53
22	†	†	†	94	92	62	51
24	†	†	†	89	88	80	32

* Each value represents the mean of the hypocotyl lengths as a % of control. † Not tested.

Table 3. Effects of 4-homoisotwistanols on the germination of lettuce seeds

	Concentration (μg/ml)					
Compound*	3.1	6.2	12.5	25	50	100
7	73	68	50	45	33	16
9	†	91	40	16	5	0
11	32	17	7	0	0	0
14	†	†	†	75	35	10
22	†	95	66	25	1	0
24	†	†	76	62	23	15

* Each value represents the mean of the number of germinated seeds as a % of control. † Not tested.

It is interesting to note that the inhibitory action of these compounds on lettuce seed germination proved to be reversible. Seeds which had not germinated after 4 days in the presence of the hydroxy compounds, after removal and washing with water, germinated in 4 hr if incubated in the absence of any test compound.

Table 4. Effects of 4-homoisotwistanols on the adventitious root formation of kidney bean cuttings

	Concentration (μg/ml)		
Compound*	1	10	100
7	116	115	193
9	121	140	250
11	82	68	120
14	105	122	166
22	102	116	145
24	111	139	255

* Each value represents the mean of the number of the roots as a % of control.

Effect on the adventitious root formation of kidney bean cuttings. The effects of test compounds on the adventitious root formation of kidney bean cuttings are shown in Table 4. All the hydroxy derivatives except **11** increased the number of roots in the hypocotyls with increasing concentration. However, they all inhibited root formation at the lowest part of the cuttings at 100 μg/ml, especially **11** which completely inhibited root formation of the part immersed in the solution, roots only being formed at boundary between the air and the solution. Nevertheless, application of these compounds did not show any pronounced effect on the size and colour of the leaves developed.

DISCUSSION

Sesquiterpenoids are now regarded as one of the most important groups of biologically active compounds. In view of the structural similarity between 4-homoisotwistane (**3**) and the sesquiterpenoids patchouli alcohol and seychellene, it was expected that derivatives of 3 would possess some biological activity. This was indeed well demonstrated in our previous study [1] on antiviral activity of some derivatives of 3 as well as in the present study.

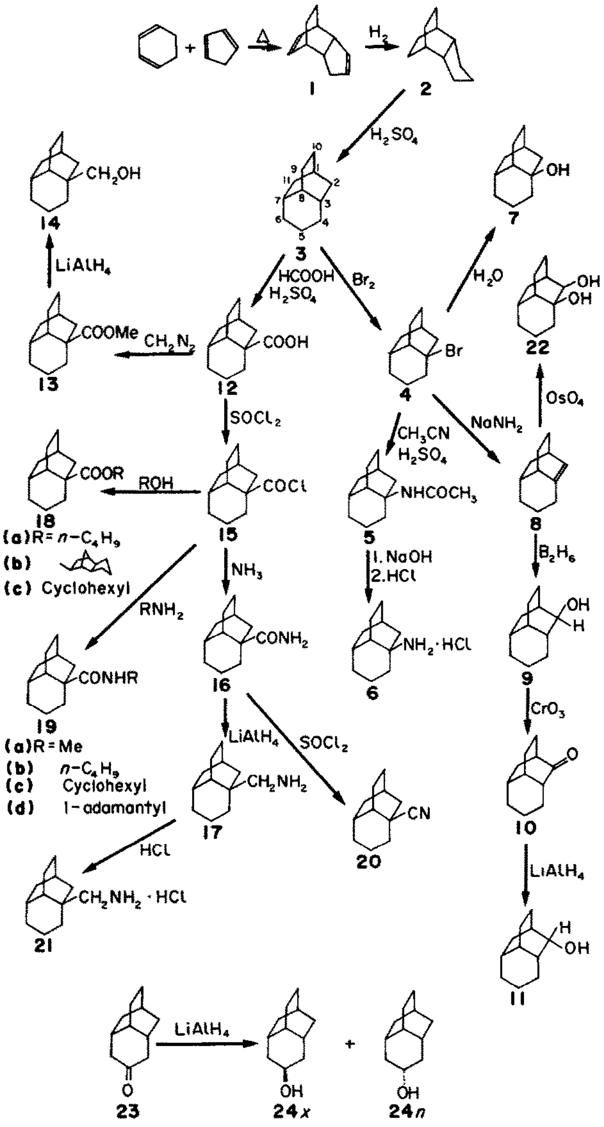
Among these derivatives, the compounds in which a hydroxy group is attached directly to the 2- or 3-position of **3** showed a very strong retardant activity on the growth of cucumber seedlings. It should be noted that the seedlings were not killed by treatment with these compounds. These compounds were also found to strongly retard the germination of lettuce seeds, but here again the seeds were not killed. Although these compounds increased the number of adventitious roots in kidney bean cuttings, they inhibited root formation at the lowest part of the cuttings [11].

We cannot conclude, from the results so far obtained, that there is any definite structure–activity relationship between the 4-homoisotwistanols tested here, but the high retardant growth activity of the hydroxy derivatives is noteworthy.

EXPERIMENTAL

Preparation of compounds. Preparation of 4-homoisotwistane (**3**) and its derivatives are summarized in Scheme 1. Preparation of the compounds listed in Table 1 as well as 2-hydroxy-4-homoisotwistanes (**9**, **11**) has been reported in detail in previous papers [1, 2, 8]. Exo-2,3-dihydroxy-4-homoisotwistane (**22**) was prepared by the reaction of the bridgehead olefin (**8**) [2b] with OsO_4 in the presence of pyridine 5-Hydroxy-4-homoisotwistane (**24**) was prepared by $LiAlH_4$ reduction of 4-homoisotwistan-5-one (**23**) [9] which had been obtained through the cyclization of the diazoketone of the Diels–Alder adduct of cyclohexa-1,3-diene and methyl acrylate. All mp's are uncorrected. Determination of IR, ^{13}C NMR and MS, and conventional and preparative GLC were carried out on the same instruments used in the previous study [1].

Exo 2,3-dihydroxy-4-homoisotwistane (**22**). A soln of 0.58 g (3.9 mmol) of **8** in 20 ml dry Et_2O was added dropwise to a soln of 1.0 g (3.9 mmol) OsO_4 and 0.7 ml Py in 20 ml dry Et_2O at 0° over a period of 15 min. Immediate formation of brown crystals was observed. The reaction was run for further 2 hr at 0°. Crystals were filtered, washed with Et_2O, and left to dry. Crystals were then added to a soln of 2.0 g mannitol in 20 ml 10% KOH at ambient temp. and the solution was extracted with 20 ml portions CH_2Cl_2, and the combined extracts were dried (Na_2SO_4). Concentration of the soln afforded the colouress crystalline diol **22** which was purified by preparative GLC to give 0.50 g (70% yield) of pure material. mp 146–147° (sealed tube). IR (nujol): 3340, 1050, cm^{-1}. MS *m/e* (rel. int.), 182 (M$^+$, 78), 164 (38), 133 (45), 112 (34), 110 (50), 108 (38), 107 (38), 104 (40), 97 (100), 95 (43), 93 (49), 91 (61), 84 (79), 81 (44), 80 (40), 79 (80), 77 (34), 67 (58), 55 (60), 53 (30), 41 (82). ^{13}C NMR (15.03 M Hz, CDCl$_3$): δ_c 17.0 (*t*), 18.5 (*t*), 19.5 (*t*), 28.8 (*t*), 30.9 (*t*), 32.2 (*d*), 32.6 (*d*), 38.9 (*t*), 39.9 (*d*), 69.6 (*s*), 71.6 (*d*).



Scheme 1. Synthesis of 4-homoisotwistane derivatives.

5-Hydroxy-4-homoisotwistane (**24**). 0.92 g (5.6 mmol) of 4-homoisotwistan-5-one (**23**) [9] in 10 ml dry Et$_2$O was added in a period of 15 min to 0.15 g (3.9 mmol) of LiAlH$_4$ and 10 ml dry Et$_2$O kept under gentle reflux. The reaction was refluxed for further 2 hr. After unreacted LiAlH$_4$ was destroyed by MeOH and H$_2$O, the mixture was acidified with 2% HCl. The organic layer was separated, and the aq layer was extracted with 3 × 30 ml Et$_2$O. Combined extracts were washed (H$_2$O) dried (Na$_2$SO$_4$) and evaporated to give 0.75 g (81% yield) of crude **24**. GLC of this product gave two peaks (83/17) and both compounds were tricycloundecanol having molecular weight 166 as determined by MS and IR. In addition, Jones oxidation of the mixture of these alcohols gave only the original ketone **23**. This result, together with the established preferential exo-attack of LiAlH$_4$ [10], enabled the assignment of the major component to the endo-isomer (24n). The endo- and exo-isomer were separable by preparative GLC.

5-Endo-hydroxy-4-homoisotwistane (**24n**). mp 97–98° (sealed tube). IR (Nujol) 3270, 1100, 1085, 990, 935 cm^{-1}. MS *m/e* (rel. int.): 166 (M$^+$, 12), 148 (100), 120 (31), 119 (37), 92 (34), 91 (41), 81 (35), 80 (98), 79 (80), 41 (41). ^{13}C NMR (15.03M Hz, CDCl$_3$): δ_c 25.1 (*d*, 1), 26.1 (*t*, 1), 26.8 (*t*, 1), 29.4 (*d*, 2), 32 0 (*d*, 1), 32.2 (*t*, 2), 39.4 (*t*, 2), 67 2 (*d*, 1).

5-Exo-hydroxy-4-homoisotwistane (**24x**), mp 101–102° (sealed tube). IR (Nujol) 3250, 1220, 1085, 1070, 1060, 1010 cm^{-1}. MS *m/e* (rel. int.): 166 (M$^+$, 22), 148 (100), 120 (29), 119 (75), 93 (32), 92 (41), 91 (36), 81 (33), 80 (51), 79 (61), 67 (31), 41 (40). ^{13}C NMR (15.03M Hz, CDCl$_3$): δ_c 24.0 (1), 25.7 (1), 25.9 (1), 31.3 (2), 32.2 (3), 41.8 (2), 64.2 (1). Fine structure of the signals of the exo-isomer could not be observed because of its small sample size.

Evaluation of plant growth retardant activities. Effect on cucumber seedlings. Seeds of cucumber (var. Fushinari) were placed on filter paper in a petri dish and moistened with H$_2$O. After 30 hr incubation in darkness at 25°, the root reached an average length of 1 mm. Sets of 10 seeds were placed in 5 ml soln containing the test compound and 0.05% (w/w) surfactant (Tween 80/Span 80 = 30/70) and kept at 25° in darkness. Hypocotyl lengths were measured after 7 days incubation.

Effect on the germination of lettuce seeds. 50 Seeds (var. Great Lakes 54) were placed in a petri dish (6 × 1.5 cm) on filter paper moistened with 1.5 ml test soln containing 0.05% surfactant (Tween 80/Span 80 = 30/70). These petri dishes were kept at 25° under "National Homolux" fluorescent tubes (*ca* 2800 lx) for 96 hr and the number of germinated seeds counted.

Effect on the adventitious root formation of kidney bean cutting. A cutting consisting of 3.0 cm of hypocotyl, about 1 cm of the epicotyl, a pair of primary leaves, and a small apical bud was taken off from light-grown (*ca* 2800 lx, 25°) 7-day old seedlings. The basal parts of 10 pieces of the cutting were dipped into a 10 ml test soln for 48 hr. After washing the basal parts with H$_2$O, the cuttings were transferred to H$_2$O and incubated under "National Homolux" fluorescent tubes (*ca* 2800 lx) at 25° throughout the experiment. The number of roots protruded from hypocotyl of the cutting was counted after 7 days incubation.

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