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SESQUITERPENOIDS FROM *CYPERUS ROTUNDUS*

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Key Word Index—*Cyperus rotundus*; Cyperaceae; sesquiterpenoid; secoeudesmanolide; cyperolone; synthesis; antibacterial activity.

Abstract—New sesquiterpenoids with 4,5-secoeudesmane and cyperolone carbon skeletons were isolated from *Cyperus rotundus*, and characterized by spectroscopic analysis. The structures were confirmed by synthesis from (+)-dihydrocarvone and (+)-cyperolone. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

The constituents of the dry root of *Cyperous rotundus* L., which is used as a traditional medicine for the treatment of stomach and bowel disorders, have been widely investigated [1–9]. Although many kinds of terpenoids have been isolated from the species, the structures differ depending on the plant's origin. Our interest in antibacterial substances led us to an examination of the plant, collected from Yorishima Island in the Seto Inland Sea in Japan. In this paper, we report on the characterization of three new sesquiterpenoids (**1**–**3**) by spectroscopic analysis and their chemical synthesis from known natural products.

RESULTS AND DISCUSSION

The methanol extract from the root of *C. rotundus* was concentrated and partitioned between hexane and water. Antibacterial activity was observed in the hexane-soluble part, from which the 4,5-secoeudesmanolide (+)-**1**, its epimer (+)-**2**, and cyclic acetal (+)-**3** were obtained as new compounds, along with six previously known terpenoids: cyperolone (**4**), mustakone (**5**) and four eudesman-type sesquiterpenoids (**6**–**9**).

Compounds (+)-**1** and (+)-**2** were isolated as a diastereomeric mixture and could be separated by silver nitrate-precoated TLC. The molecular formula of the major isomer, (+)-**1**, was determined to be $C_{15}H_{24}O_2$ by HREI-MS spectrometry. The IR spectrum showed the presence of a ketone group and a double bond. The ^1H NMR spectrum showed three methyl groups as singlets at δ 1.25, 1.74, and 2.14 for

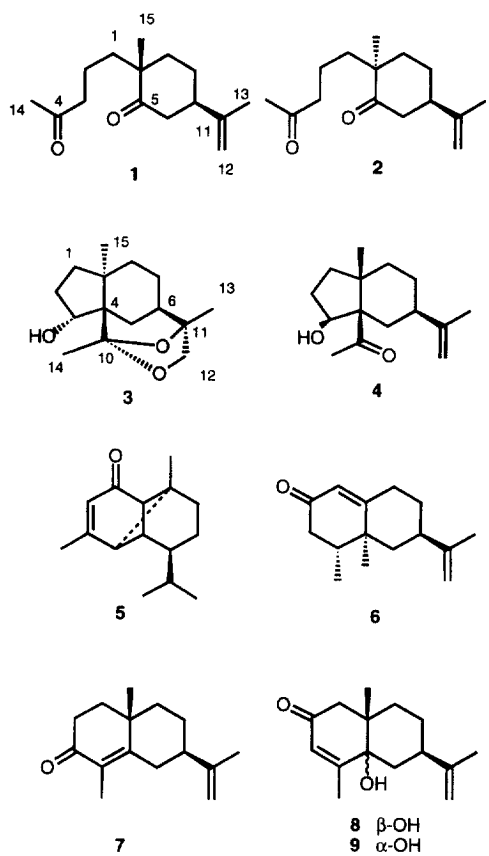

Figure 1.

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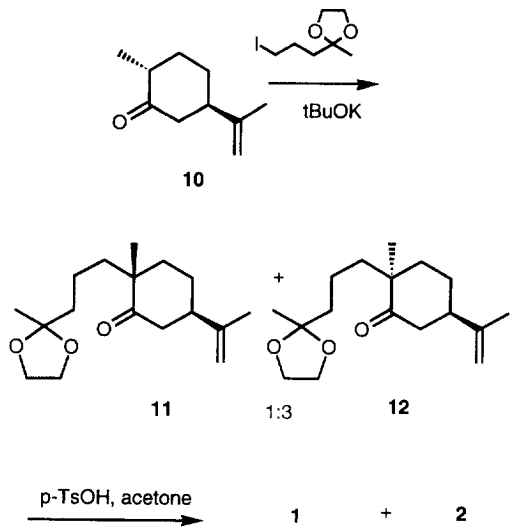
H-15, H-13 and H-14, respectively. Two olefin protons as broad singlets at δ 4.72 and 4.48 were assigned to H-12. The ^{13}C NMR spectrum showed signals for 15 carbon atoms corresponding to three CH_3, seven CH_2, one CH, two carbonyl carbons and two sp^2 carbons. The multiplicities were assigned by DEPT experiments. Fragment ion peaks at *m/z* 43 and 152 in the mass spectrum indicated a 4-oxopentyl group as the side chain. All of the signals in the ^{13}C NMR spectrum were assigned with the assistance of ^1H-^1H, ^1H-^{13}COST and HMBC experiments (Table 1).

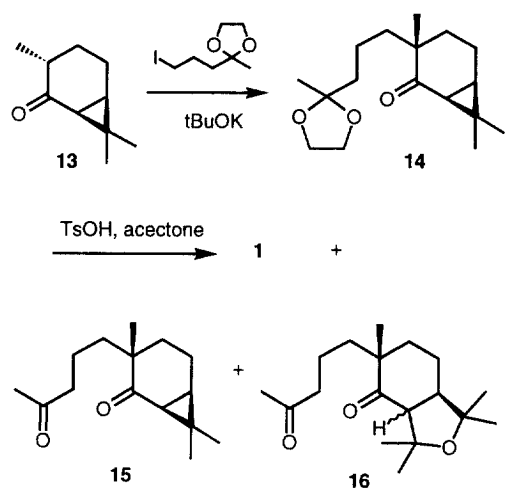
Compound (+)-**2** was obtained as the minor isomer. Its IR, EIMS, HR-EIMS spectra were quite similar to those for (+)-**1**. Obvious differences in the ^1H NMR spectrum were the chemical shifts of the H-15 (δ 1.04) and H-14 (δ 2.13) methyl groups. The signals in the ^{13}C NMR spectrum were assigned by comparison with those of (+)-**1**.

Because the stereochemistry of both compounds remained unknown, (+)-**1** and (+)-**2** were synthesized starting from (+)-dihydrocarvone (**10**) (Scheme 1). Treatment of (+)-**10** with 2-(3-iodopropyl)-2-methyl-1,3-dioxolane [10] in the presence of potassium *t*-butoxide gave a 1 : 3 epimeric mixture of **11** and **12**. It is well known that axial attack is preferred in the alkylation of **10** [11–13]; thus the major product was assumed to be **12**. Without separation of the epimers, the acetal was removed with *p*-TsOH in refluxing acetone, affording a 1 : 3 mixture of (+)-**1** and (+)-**2**. The IR, MS, ^1H NMR and ^{13}C NMR spectra of synthetic (+)-**1** and (+)-**2** were identical with those of the natural products as well as the optical rotations.

The stereochemistries were further confirmed by stereospecific synthesis of (+)-**1** *via* alkylation of 2-carone (**13**) (Scheme 2). Alkylation of **13**, derived from (+)-**10** [14], occurred only from the opposite side to the cyclopropane ring [15] giving **14** as the sole product. Treatment of **14** with *p*-TsOH in acetone at 50 gave (+)-**1**, accompanied by **15** and **16**.



Scheme. 1.



Scheme. 2.

There are two reports regarding the related 4,5-secoeudesmanolide **17** [16, 17], which is believed to be produced *via* oxidative cleavage of the co-occurring eudesmanolide **18**. Although we did not isolate the precursors of (+)-**1** and (+)-**2**, they probably originate from 7β,10β- and 7β,10α-selina-4,11-diene.

The molecular formula of (+)-**3** was determined to

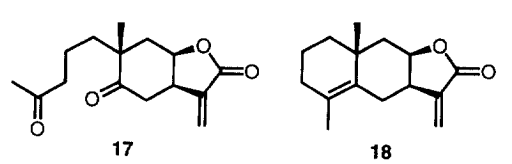
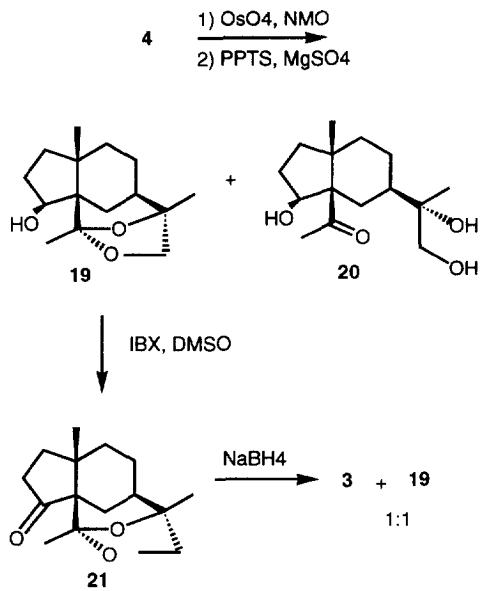


Figure 2.

Table 1. ^{13}C NMR spectral data of compounds **1**–**3** (100 MHz, $CDCl_3$)

C	1	2	3
1	37.2	38.1	35.8
2	18.1	18.0	27.9
3	45.7	43.6	74.3
4	209.0	208.3	52.7
5	215.2	215.3	25.5
6	44.2	43.4	36.3
7	43.2	46.3	24.9
8	26.0	26.0	33.7
9	36.2	36.5	44.1
10	47.3	48.0	111.8
11	147.4	147.5	84.5
12	109.9	109.8	74.8
13	20.7	20.6	19.3
14	29.9	29.9	23.0
15	23.1	22.0	29.2



Scheme. 3.

be $C_{15}H_{24}O_3$ by HREI-MS spectrometry. The IR spectrum showed the presence of a hydroxyl group, which was acetylated to give the corresponding acetate. The ^1H NMR spectrum showed three methyl groups at δ 1.02 as a doublet and δ 1.34 and 1.58 as singlets. Signals for H-12 was observed at δ 3.39 and 3.85 as doublets in addition to a triplet at δ 4.44 for H-3. The presence of six methylene groups, one of which was adjacent to oxygen, one methine group adjacent to the hydroxyl group, and four quaternary carbons were supported by the ^{13}C NMR spectrum and DEPT experiment. A characteristic singlet signal at δ 111.8 suggested the presence of an acetal moiety. The carbons were assigned as shown in Table 1 with the assistance of ^1H-^1H, ^{13}C-^1H COSY, HMBC and NOESY experiments as well as comparison with the data of the known compound (+)-cyperolone (**4**).

In order to confirm the structure, we transformed (+)-**4** into (+)-**3** in four steps (Scheme 3). Dihydroxylation of (+)-**4** with a catalytic amount of osmium tetroxide and *N*-methylmorphilin-*N*-oxide, followed by acidic treatment, gave **19** and **20** in a 2 : 1 ratio. Only the major diol, which was hydroxylated from the upper side, could cyclize to the tetracyclic acetal. IBX oxidation [18] of the alcohol **19** gave a high yield of ketone **21**. Finally, reduction of **21** with sodium borohydride gave the desired product (+)-**3** and epimeric alcohol **19** in a 1 : 1 ratio. The physical and spectroscopic properties of synthetic (+)-**3** were identical with those of the natural product, as well as the optical rotation.

The antibacterial activities of (+)-**1**, (+)-**2**, (+)-**3**, **15** and **16** against *Escherichia coli* and *Bacillus subtilis* were assayed by the paper disk method. Although (+)-**3** possessed moderate activity against *B. subtilis* at a concentration of 0.5 mg/disk (inhibition zone diameter ca 12.0 mm, paper disk : ϕ 8.0 mm), the other compounds did not show notable activities.

EXPERIMENTAL

General

Optical rotations: 0.2 or 0.5 dm cells at ambient temp.; ^1H and ^{13}C NMR: 400 and 100 MHz, respectively.

Plant material

Roots of *Cyperus rotundus* L. were collected in June 1994, in Yorishima, Okayama, Japan. The voucher specimen was deposited at the Department of Applied Science, Okayama University of Science.

Extraction and fractionation

The roots were extracted with MeOH and the crude extract (210.5 g) was partitioned between hexane and water. The hexane layer was coned and the residue (16.4 g) was sepd into 13 fractions by CC on silica gel using $CHCl_3$–Me_2CO (9 : 1). Fr. 5 (3.9 g) was chromatographed on silica gel using $CHCl_3$–Me_2CO (20 : 1), then with hexane–EtOAc (6 : 1) to give a mixture of (+)-**1** and (+)-**2** (18.8 mg). They were sepd by silver nitrate-precoated TLC, using $CHCl_3$ as solvent. (+)-**3** (17.5 mg) was obtained from fr. 8 by CC on silica gel using benzene–EtOAc (4 : 1), hexane–EtOAc (1 : 1) and then $CHCl_3$–Me_2CO (9 : 1).

2α-(5-oxopentyl)-2β-Methyl-5β-isopropenylcyclohexanone (**1**). Oil; $[\alpha]_D^{21}$ +39 ($CHCl_3$, c 0.33); HREI-MS *m/z* 236.1801 $[M]^+$, $C_{15}H_{24}O_2$ requires 236.1775; EI-MS *m/z* (rel. int.): 236 $[M]^+$ (10), 175 (16), 152 (100) 137 (22), 123 (29), 109 (100), 95 (38), 81 (49), 67 (37), 55 (32); IR ν_{max} cm^{-1}: 2938 (C=C—H), 1703 (C=O), 1655 (C=C); ^1H NMR: δ 1.14 (3H, *s*, H-15), 1.74 (3H, *brs*, H-13), 2.14 (3H, *s*, H-14), 2.45 (2H, *t*, *J* = 6.8, H-3), 2.51 (1H, *dd*, *J* = 12.9, 14.9, H-6), 4.72 (1H, *brs*, H-12), 4.78 (1H, *brs*, H-12); ^{13}C NMR: Table 1.

2β(5-oxopentyl)-2β-Methyl-5β-isopropenylcyclohexanone (**2**). Oil; $[\alpha]_D^{21}$ +90 ($CHCl_3$, c 0.21); HREI-MS *m/z* 236.1755 $[M]^+$, $C_{15}H_{24}O_2$ requires 236.1775; EI-MS *m/z* (rel. int.): 236 $[M]^+$ (17), 175 (9), 152 (100), 137 (26), 123 (38), 109 (100), 95 (48), 81 (63), 67 (46), 55 (34); IR ν_{max} cm^{-1}: 2940 (C=C—H), 1704 (C=O), 1647 (C=C); ^1H NMR: δ 1.04 (3H, *s*, H-15); 1.36 (1H, *dd*, *J* = 12.7, 3.7, H-2), 1.75 (3H, *brs*, H-13), 1.87 (1H, *dt*, *J* = 13.6, 3.7, H-8), 2.13 (3H, *s*, H-14), 2.35 (1H, *dd*, *J* = 13.7, 3.7, H-6), 2.52 (1H, *t*, *J* = 13.7, H-6), 4.73 (1H, *brs*, H-12), 4.77 (1H, *brs*, H-12); ^{13}C NMR: Table 1.

Tetracyclic acetal with the cyperolone skeleton (**3**). Oil; $[\alpha]_D^{21}$ +15° ($CHCl_3$, c 0.04); HREI-MS *m/z* 252.1729 $[M]^+$, $C_{15}H_{24}O_3$ requires 252.1724; IR ν_{max} cm^{-1}: 3575 (OH); ^1H NMR: δ 1.02 (3H, *d*, *J* = 0.7, H-15), 1.34 (3H, *s*, H-13), 1.58 (3H, *s*, H-14), 3.39

(1H, *d*, $J = 6.6$, H-12), 3.85 (1H, *d*, $J = 6.6$, H-12), 4.44 (1H, *t*, $J = 8.4$, H-3); ^{13}C NMR: Table 1.

Derivation of (+)-**1** *and* (+)-**2** *from dihydrocarvone.* To a soln of dihydrocarvone (119 mg) in THF (2 ml) was added *t*-BuOK in THF (1.0 M, 0.936 ml) at 0°. After stirring for 25 min, a soln of 2-(3-iodopropyl)-2-methyl-1,3-dioxolane (200 mg) in THF (2 ml) was added to the mixture at 0°. The reaction temp. was raised to rt, and stirring was continued for 1.5 h. The reaction was quenched with satd NH_4Cl, and the mixture was extracted with hexane. The organic layer was dried (Na_2SO_4), evaporated and chromatographed on silica gel (hexane–EtOAc 10 : 1) to give **11** and **12** (80 mg, 37% yield) as an oil.

A soln of **11** and **12** (166 mg) in Me_2CO (5 ml) was treated with *p*-TsOH (5 mg) at 50° for 2 h. After addition of satd $NaHCO_3$, the solvent was removed. The residue was extracted with CH_2Cl_2, and the organic layer was dried (Na_2SO_4), and evaporated. Purification by CC gave a mixture of (+)-**1** and (+)-**2** (45 mg, 32%) as a 1 : 3 diasteromeric mixture, which was run on silver nitrate-precoated TLC plate to give (+)-**1** and (+)-**2**.

Derivation of (+)-**1** *from* 2-*carone.* The reaction was carried out under conditions similar to those used for the synthesis of **11** and **12**, using **13** (100 mg), 2-(3-iodopropyl)-2-methyl-1,3-dioxolane (169 mg) and *t*-BuOK in THF (1.0 M, 0.792 ml). Workup and purification by CC (hexane–EtOAc 5 : 1) gave the product **14** (130 mg, 70% yield).

The acetal **14** (300 mg) in Me_2CO was treated with a catalytic amount of *p*-TsOH at 50° for 40 min. The reaction was neutralized with satd $NaHCO_3$ and the solvent was removed. CC and repeated prep. TLC using hexane–EtOAc or hexane–Me_2CO as solvents gave the desired diketone (+)-**1** (24 mg, 10%), the bicyclic diketone **15** (149 mg, 59%) and the Me_2CO adduct **16** (32 mg, 10%). **15**: oil; $[\alpha]_D^{21}$ −118° ($CHCl_3$, c 0.24); EI-MS *m/z* (rel. int.): 236 $[M]^+$ (12), 175 (35), 152 (65), 151 (54), 110 (49), 95 (82), 82 (100), 67 (54), 43 (28), 28 (34); UV ($CHCl_3$) λ_{max} 238.8 nm ($\varepsilon = 587.6$); IR ν_{max} cm^{-1}: 2870 (C═C—H), 1713 (C═C); ^1H NMR: δ 0.96 (3H, *s*), 1.07 (3H, *s*), 1.12 (3H, *s*), 2.13 (3H, *s*), 2.41 (2H, *dt*, $J = 6.7$, 1.8); ^{13}C NMR: δ 16.0 (*t*), 17.4 (*q*), 18.0 (*t*), 20.6 (*q*), 23.0 (*d*), 25.1 (*d*), 29.7 (*q*), 29.9 (*q*), 32.1 (*d*), 32.2 (*t*), 36.4 (*t*), 43.8 (*t*), 45.8 (*s*), 208.5 (*s*), 213.5 (*s*). **16**: oil; $[\alpha]_D^{21}$ −82° ($CHCl_3$, c 0.10); IR ν_{max} cm^{-1}: 1714 (C═O), 1688 (C═O); ^1H NMR: δ 1.08 (3H, *s*), 1.13 (3H, *s*), 1.25 (3H, *s*), 1.27 (3H, *s*), 1.47 (3H, *s*) 2.13 (3H, *s*), 2.18 (3H, *ddd*, $J = 13.2$, 8.8, 4.4), 2.97 (1H, *dd*, $J = 6.8$, 0.8); ^{13}C NMR: δ 18.5 (*t*), 21.5 (*t*), 25.1 (*q*), 25.4 (*q*), 28.8 (*q*), 30.0 (*q*), 30.9 (*q*), 33.7 (*q*), 34.4 (*t*), 40.7 (*t*), 43.9 (*t*), 46.7 (*s*), 50.5 (*d*), 60.4 (*d*), 82.0 (*s*), 83.3 (*s*), 208.6 (*s*), 217.8 (*s*).

Dihydroxylation of cyperolone (**4**). **4** (5.1 mg) was treated with OsO_4 (1 mg) and *N*-methylmorpholin-*N*-oxide (0.03 ml) in THF–water (2 : 1, 1 ml) at room temp. The THF was evaporated and the residue was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated. Prep. TLC using CH_2Cl_2–MeOH (10 : 1) gave **19** (2.7 mg, 50%) and **20** (1.6 mg, 27%). **19**: Oil; $[\alpha]_D^{21}$ +43° ($CHCl_3$ c, 0.12); IR ν_{max} cm^{-1}: 3418, 3027 (OH); ^1H NMR: δ 1.20 (3H, *s*), 1.25 (3H, *s*), 1.68 (3H, *s*), 3.35 (1H, *d*, $J = 6.6$), 3.75 (1H, *d*, $J = 6.6$), 3.79 (1H, *q*, $J = 3.6$); ^{13}C NMR: δ 19.4, 23.9, 26.8, 29.1, 32.4, 32.7, 34.5, 36.0, 37.2, 45.1, 68.6, 74.6, 80.8, 83.6, 112.1. **20**: Oil; $[\alpha]_D^{21}$ −10.4° ($CHCl_3$, c 0.115); IR ν_{max} cm^{-1}: 3410, 3040 (OH). 1719 (C═O); ^1H NMR: δ 0.89 (3H, *s*), 1.03 (3H. *s*), 2.11 (3H, *s*), 3.32 (1H, *d*, $J = 11.5$), 3.64 (1H, *d*, $J = 11.5$), 4.43 (1H, *q*, $J = 5.5$); ^{13}C NMR: δ 19.4, 21.8, 22.4, 26.9, 30.7, 31.3, 37.5, 37.8, 38.3, 42.7, 64.3. 67.7, 74.7, 76.1, 215.0.

Oxidation of alcohol (+)-**19**. A soln of (+)-**19** (2.2 mg) and *o*-iodoxybenzoic acid (6.1 mg) in DMSO was heated at 50° for 3 h. After addition of water, the mixture was extracted with Et_2O. The organic layer was dried (Na_2SO_4) and evaporated. CC on silica gel (hexane–EtOAc 3 : 1) gave ketone **21** (2.2 mg, 91%) Oil; $[\alpha]_D^{21}$ −62° ($CHCl_3$ c 0.12); HREI-MS *m/z* 250.1572 $[M]^+$, $C_{15}H_{22}O_3$ requires 250.1568; IR ν_{max} cm^{-1}: 1730 (C═O); ^1H NMR δ 1.04 (3H, *s*), 1.29 (3H, *s*), 1.50 (3H, *s*), 3.40 (1H, *d*, $J = 6.8$), 3.78 (1H, *d*, $J = 6.8$); ^{13}C NMR: δ 19.1, 23.2, 24.8, 28.8, 29.7, 30.7, 32.6, 33.0, 35.4, 42.6, 61.1, 75.1, 84.1, 109.1, 218.6.

Reduction of ketone **21**. **21** (6 mg) was treated with $NaBH_4$ (10 mg) in MeOH (2.5 ml) at 0° for 3 h. After quenching with 1% HCl, the mixture was neutralized with satd $NaHCO_3$, then extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated. Prep. TLC using hexane–EtOAc (3 : 1) as solvent gave (+)-**3** (2.5 mg, 34%) and **19** (2.2 mg, 34%).

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