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# REARRANGED CUPARANE-TYPE SESQUI- AND CLERODANE-TYPE DITERPENOIDS FROM THE LIVERWORT *DEMOTARISIA LINGUIFOLIA*

FUMIHIRO NAGASHIMA and YOSHINORI ASAKAWA

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan

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**Key Word Index**—*Demotarisia linguifolia*; Jungermanniaceae; Hepaticae; rearranged cuparane-type sesquiterpenoid; clerodane-type diterpenoid.

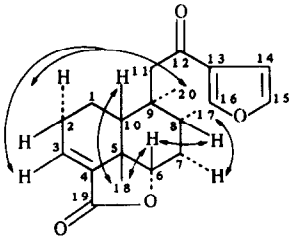
**Abstract**—A new rearranged cuparane-type sesquiterpenoid and two new clerodane-type diterpenoids, linguifolide and dihydrolinguifolide, have been isolated from the liverwort *Demotarisia linguifolia* and their structures established by extensive NMR spectroscopy.

## INTRODUCTION

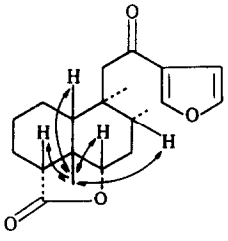
A number of the species belonging to the Jungermanniaceae are rich sources of sesqui- and diterpenoids [1]. The genus *Demotarisia* belonging to the Jungermanniaceae contains only one species, *D. linguifolia*. We have now investigated the chemical constituents of *D. linguifolia* and isolated a new rearranged cuparane-type sesquiterpenoid and two new clerodane-type diterpenoids. In this paper, we report the characterization of these compounds.

## RESULTS AND DISCUSSION

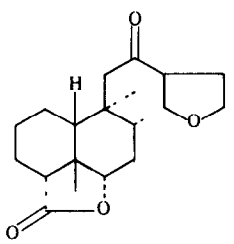
The liverwort *D. linguifolia* was extracted with ether and then methanol. Both extracts were combined and then chromatographed over silica gel and Sephadex LH-20 to afford a rearranged cuparane-type sesquiterpene (**3**) and two clerodane-type diterpenes named linguifolide (**1**) and dihydrolinguifolide (**2**). Linguifolide (**1**) had the molecular formula $C_{20}H_{24}O_4$ (*m/z* 328.1674) indicating nine degrees of unsaturation. Its IR spectrum showed the presence of a furan ring (3170, 3140, 1515 and 890 $cm^{-1}$), a lactone (1760 $cm^{-1}$) and a conjugated carbonyl group (1690 $cm^{-1}$). The $^1H$ NMR spectrum (Table 1) contained the signals of two tertiary methyl, one secondary methyl, a methine proton bearing an oxygen [$\delta$4.34 (1H, *dd*, $J$ = 11.2, 7.3 Hz)], a $\beta$-substituted furan ring [$\delta$6.70 (1H, *dd*, $J$ = 2.0, 1.0 Hz), 7.40 (1H, *t*, $J$ = 2.0 Hz) and 8.01 (1H, *s*)] and a trisubstituted olefinic proton [$\delta$6.73 (1H, *t*, $J$ = 3.9 Hz)]. The $^{13}C$ NMR spectrum displayed 20 carbons (Table 2); three methyls, four methylenes, two methines, a methine carbon bearing an oxygen ($\delta$85.1), furan ring ($\delta$108.5, 129.1, 144.3 and 146.8), two olefinic carbons ($\delta$133.7 and 135.7), two carbonyl carbons ($\delta$169.8 and 194.0) and two quarternary carbons. These spectral features disclosed that **1** must be a furanoditerpenelactone. The $^1H$–$^1H$ and $^1H$–$^{13}C$ COSYs of **1** were extensively examined to clarify the connectivity of each proton in **1**. The olefinic proton at $\delta$6.73 (H-3) was coupled with a methylene proton at $\delta$2.33 (H-2, 2H). The methylene proton was correlated with a methylene proton at $\delta$1.89 (H-1, 2H), which was coupled with a methine proton at $\delta$2.40 (H-10). The methine bearing an oxygen at $\delta$4.34 (H-6) was correlated with a non-equivalent methylene proton at $\delta$1.13–1.22 and 1.81 (H-7), one of which was coupled with a methine proton at $\delta$2.12 (H-8). The methine proton was correlated with a secondary methyl at $\delta$0.81 (H-17). Furthermore, the proton at $\delta$2.64 (H-11) was coupled with only a proton at $\delta$2.88 (H-11). These results led to the partial structures; (A) –CH(10)–$CH_2$(1)–$CH_2$(2)–CH(3)=C(4)–, and (B) Me(17)–CH(8)–$CH_2$(7)–CH(6)–O– and (C) –$CH_2$(11)–. The long-range $^1H$–$^{13}C$ COSY spectrum (Table 3) of **1** indicated that the tertiary methyl proton (H-20) was correlated with the two methine carbons (C-8 and 10), the methylene carbon (C-11) and the quarternary carbon (C-9). Another tertiary methyl (H-18) was correlated with the methine carbon at $\delta$41.5 (C-10) and 85.1 (C-6) and the quarternary carbon at $\delta$39.6 (C-5) and 133.7 (C-4). Furthermore, the non-equivalent methylene protons (H-11)



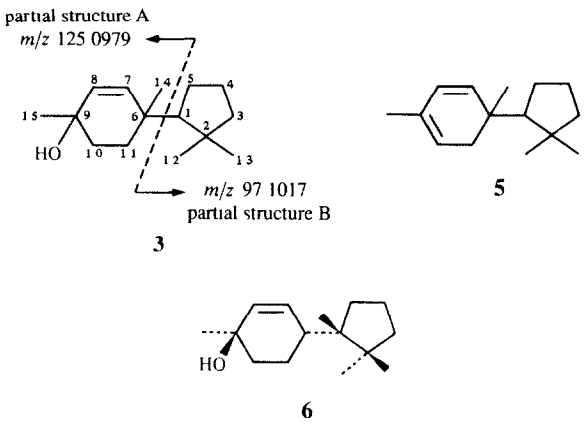
**1** ( NOE )



**2** ( NOE )



**4**




3 5 6

Table 1. $^1$H NMR data of compounds **1** and **2** (400 MHz, $CDCl_3$, TMS)

| H | 1 | 2 |
|---|---|---|
| 1 | 1 89 *m* | 1 02 *m* |
| | | 1 44 *m* |
| 2 | 2 33 *m* | 1 64–1.77[a] *m* |
| | | 1 98–2.07[b] *m* |
| 3 | 6 73 *t* (3 9) | 1 19 *m* |
| | | 1.64–1.77[a] *m* |
| 4 | | 2.17 *dd* (7.3, 2.0) |
| 6 | 4.34 *dd* (11 2, 7 3) | 4 20 *dd* (9.3, 2 9) |
| 7 | 1.13–1.22 *m* | 1 64–1.77[a] *m* |
| | 1.81 *dq* (13 2, 6 8, 2.0) | 1.98–2.07[b] *m* |
| 8 | 2 12 *d six* (13 7, 13.7, 6.8, 2 4) | 2 34 *m* |
| 10 | 2 40 *m* | 1 98–2 07[b] *m* |
| 11 | 2 64 *d* (17 1) | 2 65 *d* (17 1) |
| | 2 88 *d* (17 1) | 2 78 *d* (17 1) |
| 14 | 6 70 *dd* (2 0, 1 0) | 6 65 *s* |
| 15 | 7 40 *t* (2.0) | 7.36 *t* (1 5) |
| 16 | 8 01 *s* | 7 95 *s* |
| 17 | 0 81 *d* (6 8) | 0 79 *d* (6 8) |
| 18 | 1 19 *s* | 1 31 *s* |
| 20 | 0.76 *s* | 0 80 *s* |

*Figures in parentheses are coupling constants in Hz
[a,b]Overlapped signal within the same column

Table 2 $^{13}$C NMR data of compounds **1–3** (100 MHz, $CDCl_3$)*

| C | 1 | 2 | 3 |
|---|---|---|---|
| 1 | 18 0 | 23 0 | 58 0 |
| 2 | 24.5 | 17 4 | 41 3 |
| 3 | 135 7 | 19 9 | 45 1 |
| 4 | 133 7 | 48 6 | 20 8 |
| 5 | 39 6 | 41 0 | 27 4 |
| 6 | 85 1 | 83 3 | 37.7 |
| 7 | 34 8 | 30 4 | 139 1 |
| 8 | 31 8 | 29 8 | 129.9 |
| 9 | 41 2 | 38 4 | 66.9 |
| 10 | 41 5 | 44 7 | 34.1 |
| 11 | 46 3 | 48 1 | 29.7 |
| 12 | 194 0 | 194 3 | 31 9[a] |
| 13 | 129 1 | 128 9 | 24 6[a] |
| 14 | 108 5 | 108 3 | 25 5 |
| 15 | 144 3 | 144 1 | 29 7 |
| 16 | 146 8 | 146 7 | |
| 17 | 16 0 | 15 8 | |
| 18 | 30 2 | 29 8 | |
| 19 | 169 8 | 179 2 | |
| 20 | 15 9 | 17 1 | |

*All assignments were confirmed by INEPT, $^1$H–$^{13}$C COSY and long-range $^1$H–$^{13}$C COSY spectra
[a]May be interchanged

Table 3 Long-range correlations observed in the long-range $^1$H–$^{13}$C COSY spectra of compound **1**

| H (δ) | Correlated carbon |
|---|---|
| 3 (6 73) | 1, 5, 19 |
| 11 (2.64 and 2 88) | 8, 9, 10, 12 |
| 17 (0 81) | 7, 8 |
| 18 (1 19) | 4, 5, 6, 10 |
| 20 (0 76) | 8, 9, 10, 11 |

were correlated with two methine carbons (C-8 and 10), the quarternary carbon (C-9) and the carbonyl carbon at δ194.0 (C-12). The olefinic proton at δ6 73 (H-3) was correlated with the lactone carbonyl carbon at δ 169.8 (C-19), the methylene carbon (C-1) and the quarternary carbon (C-5) The above spectral evidence disclosed that **1** was a clerodane-type diterpene with a β-substituted furan ring conjugated with a ketone group and a conjugated γ-lactone. The relative stereochemistry of **1** was determined by NOE difference spectroscopy The NOEs were observed between (i) H-20 and H-2α, (ii) H-20 and H-3, (iii) H-17 and H-7α, (iv) H-18 and H-6β, (v) H-18 and H-10β, (vi) H-6β and H-8β. These results showed that **1** was a *cis*-clerodane-type diterpene. On the basis of the spectral evidence, the relative stereostructure of linguifolide was established to be **1**.

The $^1$H and $^{13}$C NMR data of dihydrolinguifolide (**2**), $C_{20}H_{26}O_4$ (*m/z* 330.1826), resembled those of linguifolide (**1**), except for the absence of signals due to a trisubstituted double bond. A NOE difference experiment showed the presence of the NOEs between (i) H-18 and H-4, (ii) H-18 and H-6, (iii) H-18 and H-8, (iv) H-18 and H-10. Catalytic hydrogenation of **2** gave a tetrahydro derivative (**4**), the $^1$H NMR data of which were identical to **4** prepared from **1** by hydrogenation Thus, the structure of dihydrolinguifolide was established to be **2**

High resolution mass spectrometry of **3** gave the molecular formula $C_{15}H_{26}O$ (*m/z* 222 1973) The IR, and $^1$H and $^{13}$C (Table 2) NMR spectra revealed the presence of a tertiary hydroxyl (3350 cm$^{-1}$; $\delta_C$ 66.9 *s*), a *cis*-ethylenic [$\delta_H$ 5.44, 5.59 (each 1H, *d*, $J = 10$ 3 Hz); $\delta_C$ 129.9, 139 1 (each *d*)] and four tertiary methyl groups [$\delta_H$ 0.97, 1.01, 1.14, 1 25, (each 3H, *s*); $\delta_C$ 24.6, 25 5, 29 7, 31.9 (each *q*)] together with five methylene, one methine and two quarternary carbons. These spectral data closely resembled the cuparane-type sesquiterpene alcohol, cu-

prenenol (**6**) which has been previously isolated from the liverwort *Jungermannia rosulans* [2], suggesting that compound **3** was a cuprenenol like sesquiterpenoid. Dehydration of **3** with phosphorus oxychloride in pyridine gave the sesquiterpene hydrocarbon **5**, *m/z* 204 $[M]^+$. The long-range $^1H$–$^{13}C$ COSY of **3** indicated that the tertiary methyl proton at $\delta$1.01 (H-14) was correlated with the methylene carbon (C-11), the methine carbon (C-1), the quarternary carbon at $\delta$37.7 (C-6) and the olefinic carbon at $\delta$139.1 (C-7). Another tertiary methyl proton at $\delta$1.25 (H-15) was correlated with the methylene carbon at $\delta$34.1 (C-10), the olefinic carbon at $\delta$129.9 (C-8) and with the quarternary carbon bearing a hydroxy group (C-9). In addition, two tertiary methyl protons at $\delta$0.97 (H-13) and 1.14 (H-12) assignable to C-12 and C-13 (vice versa) were correlated with the methyl carbon at $\delta$31.9 and 24.6, the methylene (C-3), the methine carbon (C-1) and with the quarternary carbon (C-2), respectively. Moreover, the fragment ions, $C_8H_{13}O$ (found 125.0979, calcd 125.0966) and $C_8H_{12}$ (found 108.0942, calcd 108.0939) and $C_7H_{13}$ (found 97.1017, calcd 97.1017) of the mass spectrum of **3** indicated the presence of two partial structures A and B [3]. Consideration of these spectral data led to the conclusion that the gross structure of **3** was depicted as the rearranged cuparane-type sesquiterpene alcohol. In order to clarify the relative stereochemistry of **3**, a NOE difference examination was carried out, however, no useful information was obtained by this method.

The genus *Demotarisia* resembles morphologically the genus *Jamesoniella* (Mizutani, M. personal communication) belonging to the Lophoziaceae. Our findings show that *J. autumnalis* produces kaurane-type diterpenoids as major components; clerodane-type diterpenoids have not been detected [4]. Thus, there is no chemical affinity between *D. linguifolia* and *J. autumnalis* although only one species of the *Jamesoniella* species has chemically been investigated.

## EXPERIMENTAL

Mps. uncorr The solvents used for spectral determinations were TMS–$CDCl_3$ [$^1$H NMR (400 MHz), $^{13}$C NMR (100 MHz)]; $CHCl_3$ (IR and $[\alpha]_D$); EtOH (UV); MeOH (CD). MeOH–$CHCl_3$ (1:1) was used for Sephadex LH-20 CC. TLC and GC were carried out as previously reported [5].

*Plant material. Demotarisia linguifolia* (DeNot.) Grolle was collected in East Malaysia, in 1987 and identified by Dr M. Mizutani. The voucher specimen is deposited at the Institute of Pharmacognosy, Tokushima Bunri University

*Extraction and isolation.* Fresh *D. linguifolia* was ground mechanically and extracted with $Et_2O$ for 1 month and MeOH for 1 month. Both extracts were combined and the crude extract (7.9 g) was chromatographed on silica gel using a *n*-hexane–EtOAc gradient. Eight fractions were collected. Fr 5 (692 mg) was rechromatographed on Sephadex LH-20 and on silica gel (*n*-hexane–EtOAc and $C_6H_6$–EtOAc) to give **3** (126 mg) oil; $[\alpha]_D$ −39.6° (*c* 4.04); HRMS: Found $[M]^+$ 222.1973; $C_{15}H_{26}O$ requires 222.1984. IR $\nu_{max}^{neat}$ cm$^{-1}$: 3350; $^1$H NMR. $\delta$0.97 (3H, *s*, H-13), 1.01 (3H, *s*, H-14), 1.14 (3H, *s*, H-12), 1.25 (3H, *s*, H-15), 2.03 (1H, *m*, H-11), 5.44 (1H, *d*, *J* = 10.3 Hz, H-8), 5.59 (1H, *d*, *J* = 10.3 Hz, H-7); $^{13}$C NMR: Table 2; EIMS *m/z* (rel. int.): 222 $[M]^+$ (6), 205 (50), 189 (14), 177 (4), 162 (8), 147 (9), 133 (10), 125 (64), 108 (100), 97 (98), 93 (64), 81 (56), 69 (23), 55 (95), 43 (96); Fr 6 (630 mg) was rechromatographed on Sephadex LH-20 and on silica gel ($C_6H_6$–EtOAc) to give a dihydrolinguifolide (**2**) (104 mg): crystals; mp 158–160°, $[\alpha]_D$ +22° (*c* 0.18); UV $\lambda_{max}$ nm (log $\varepsilon$): 203 (3.95), 251 (3.63); CD. $\Delta\varepsilon_{245\ nm}$ +0.5 (sh), $\Delta\varepsilon_{215\ nm}$ +0.9, IR $\nu_{max}^{KBr}$ cm$^{-1}$ 3170, 3140, 1770, 1675, 1150, 980, 880; $^1$H and $^{13}$C NMR (Table 1 and 2); EIMS *m/z* (rel int.) 330 $[M]^+$ (7), 220 (100), 205 (63), 175 (24), 159 (29), 133 (22), 119 (15), 110 (64), 95 (71), 79 (14), 67 (16), 55 (18), 41 (25), Fr. 7 (662 mg) was rechromatographed on Sephadex LH-20 and on silica gel ($C_6H_6$–EtOAc) to give linguifolide (**1**) (73 mg) crystals; mp 130–133°; $[\alpha]_D$ −24° (*c* 0.25); UV $\lambda_{max}$ nm (log $\varepsilon$) 215 (4.19), 253 (3.65, sh); CD. $\Delta\varepsilon_{248\ nm}$ +7.1, $\Delta\varepsilon_{210\ nm}$ −11.3; IR $\nu_{max}^{KBr}$ cm$^{-1}$. 3150, 3170, 1760, 1680, 1160, 990, 890; $^1$H and $^{13}$C NMR (Table 1 and 2); EIMS *m/z* (rel. int.): 328 $[M]^+$ (11), 313 (3), 219 (100), 203 (42), 173 (6), 149 (11), 133 (6), 125 (16), 110 (31), 95 (72), 77 (12), 67 (9), 55 (8), 41 (12)

*Hydrogenation of* **1**. Compound **1** (4 mg) in EtOH (6 ml) was hydrogenated in the presence of 5% Pd-C (10 mg) for 1 hr. Work-up as usual gave **4** (3 mg). $^1$H NMR (90 MHz): $\delta$0.82 (3H, *s*), 0.83 (3H, *d*, *J* = 6.6 Hz), 1.38 (3H, *s*), 3.14 (*m*), 3.65–3.94 (*m*), 4.28 (1H, *dd*, *J* = 8.6, 2.9 Hz)

*Hydrogenation of* **2**. Compound **2** (6 mg) in EtOH (5 ml) was hydrogenated in the same manner as described above to give a compound whose $^1$H NMR data was identical to that of **4**.

*Dehydration of* **3**. To a soln of **3** (49 mg) in pyridine (2 ml) was added $POCl_3$ (0.2 ml), and the mixture stirred overnight. The reaction mixture was poured into ice-$H_2O$ and extracted with $Et_2O$. The residue after removal of the solvent was chromatographed on silica gel (*n*-hexane) to give **5** (1 mg); $^1$H NMR: $\delta$0.96 (3H, *s*), 1.00 (3H, *s*), 1.09 (3H, *s*), 1.55 (3H, *s*), 5.33 (1H, *br s*), 5.59 (1H, *d*, *J* = 9.3 Hz), 5.67 (1H, *d*, *J* = 9.3 Hz), EIMS *m/z* (rel. int.): 204 $[M]^+$ (7), 149 (3), 119 (6), 107 (100), 97 (28), 79 (13), 69 (9), 55 (41), 41 (20).

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