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(−)-LEDOL FROM THE LIVERWORT *CEPHALOZIELLA RECURVIFOLIA* AND THE CLARIFICATION OF ITS IDENTITY

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Key Word Index—*Cephaloziella recurvifolia*; Cephaloziellaceae; liverwort; (−)-ledol; globulol; viridiflorol.

Abstract—(−)-Ledol was found in the liverwort *Cephaloziella recurvifolia* along with other known sesquiterpenes. This is the first natural occurrence of this enantiomer of ledol. This is also the first chemical study on this family. Confusing assignments for ledol, globulol and viridiflorol in the literatures are discussed in this paper.

INTRODUCTION

Liverworts are known to elaborate sesquiterpenes, diterpenes or lipophilic aromatic compounds as their major constituents. In the order Jungermanniales, the chemistry of many family species has been investigated. However, the Cephaloziellaceae is one family which has not been previously examined. Therefore, we have studied *Cephaloziella recurvifolia* collected in China. Its major constituents were identified as sesquiterpenoids and among them, (−)-ledol (**1**) is worthy of mention since its identification often appears confusing in the literature when compared with its two diastereomers, globulol (**2**) and viridiflorol (**3**).

RESULTS AND DISCUSSION

The crude oil of *C. recurvifolia* was rich in oxygenated sesquiterpenes. Among them, the major component was identified to be 3α-acetoxybicyclogermacrene (**4**) on the basis of its mass spectral fragmentation [1]. The second major compound ($[M]^+$ at *m/s* 222, $C_{15}H_{26}O$) was isolated from the 15% ethylacetate *n*-hexane eluate of the crude oil from a silica gel column. Its ^1H NMR (Table 1) clearly indicated a cyclopropyl ring (δ 0.11 *dd*, $J = 9.2$ and 9.2 Hz, 1H; 0.61 *ddd*, $J = 12.6$, 9.2 and 6.5 Hz, 1H), one secondary methyl (δ 0.92 *d*, $J = 6.7$ Hz) and three tertiary methyls (δ 0.98, 1.02 and 1.15, all *s*). Combining observations from ^{13}C-DEPT, ^{13}C–^1H COSY, HMBC NMR and mass spectral data, the skeleton of 10-hydroxyaromadendrane (**5**) was proposed for this component. Upon careful comparison of spectral data (^1H and ^{13}C NMR and mass spectra) with literature reports, ledol (**1**) was suggested for this component since both its ^1H and ^{13}C NMR data were identical to those reported for a commercial sample [2]. However, there seemed several mistakes in the identification of ledol (**1**) and viridiflorol (**3**), and wrong chemical shift assignments for globulol (**2**) in the literature.

San Feliciano *et al.* [3] described the isolation of the acetate (**6**) of an alloaromadendrane diol. After some chemical transformations, compound **3** was obtained. Nevertheless, both the ^1H and ^{13}C NMR data for this alcohol (**3**) that were reported were actually identical to those of **1** [2] although several assignments of the ^{13}C NMR shifts were in a different order. The former authors [3] claimed that the identity of **3** was ascertained after comparison of the spectral data with those of diastereomers reported in the literature [4, 5], in which only IR spectra and $[\alpha]_D$ data for the three alcohols **1–3** were provided. Koul *et al.* [6] claimed (−)-ledol to be a new enantiomer isolated from *Piper clusii*. However, neither the ^1H NMR nor the ^{13}C NMR data were identical to those for commercial ledol [2] or commercial globulol [2]. The assignment was only based on a comparison with partial ^1H NMR data (δ 0.33 and 0.72 for two protons on a cyclopropyl ring; δ 0.94, 0.98, 1.04 and 1.14 for four methyl protons) of ledol reported by Pakrshi *et al.* [7]. In the latter paper [7], the identity was assumed by direct comparison (melting-point, TLC, IR) with an authentic sample without indicating the source of the authentic sample. Furthermore, comparisons on data such as melting-point, TLC and IR certainly are not conclusive and may sometimes be doubtful. Since the alcohol isolated from the last two plant species (*Piper* sp. and *Aristolochia* sp.) [6, 7] is neither **1** nor **2**, this compound is temporarily labelled as diastereomer A, and its NMR data are compiled in Table 1 for comparison.

Compound **3** has been reported as a constituent in many essential oils and an isolate from niaouli (*Melaleuca* sp.) oils [8, 9]. In the latter case, the identification of **3** was based on data reported in ref.

Table 1. ^{13}C and ^{1}H NMR data for ledol (**1**), globulol (**2**), viridiflorol (**3**) and their diastereomer A

Atom	**1***	**2**†	**3**‡?	**3**§?	Diastereomer A‖
C-1	58.2	56.8	58.4	58.7	54.1
C-2	25.8	26.5	25.9	26.0	21.0
C-3	29.1	28.6	29.3	29.6	30.6
C-4	38.4	39.5	38.5	38.7	40.5
C-5	39.7	44.3	39.9	40.1	38.2
C-6	22.3	26.1	22.5	22.8	23.2
C-7	28.7	28.4	28.7	29.0	28.5
C-8	18.8	20.1	18.9	19.2	23.2
C-9	37.8	36.3	37.9	38.0	39.0
C-10	74.6	75.1	74.5	73.7	74.4
C-11	18.4	19.2	18.4	18.5	18.5
C-12	16.1¶	15.7¶	16.1	16.3	15.0
C-13	28.6¶	28.1¶	28.7	28.8	30.2
C-14	32.1	34.5	32.1	32.4	30.2
C-15	16.3	16.1	16.3	16.5	15.4
H-6	0.61	0.58	0.61		0.72
H-7	0.11	0.52	0.11		0.33
H-12	0.98¶	0.98¶	1.00		0.98
H-13	1.02¶	1.01¶	1.03		1.05
H-14	1.15	1.10	1.14		1.15
H-15	0.92	0.92	0.93		0.94

*Data from the present research and ref. [2].

†Data from the present research on a commercial sample and ref. [2]. Some of the ^{13}C NMR shifts in ref. [2] were assigned in wrong order.

‡Data from refs [3, 8], given for viridiflorol, but actually consistent with those of ledol except several assigned in wrong order in ref. [3].

§Data from ref. [10], given for viridiflorol, but actually consistent with those of ledol. The original data are compiled in numerical order.

‖Data from ref. [6] in which the compound was mistakenly assigned as ledol.

¶Assigned according to difference NOE observations, and ^{13}C–^{1}H COSY correlations.

[8], in which the ^{1}H and the ^{13}C NMR shift assignments of **3** (Table 1) were deduced on the basis of ^{1}H–^{1}H and ^{13}C–^{1}H COSY NMR experiments. However, actually both ^{1}H and ^{13}C NMR data for **3** listed in this paper were identical to those of **1** of a commercial sample [2]. No discussion was presented by the authors to distinguish between these two stereoisomers. In addition, the ^{1}H and ^{13}C NMR data for **2** were also included in the same paper [8]. These data were essentially identical to those obtained for our sample, which was purchased commercially, except for seven incorrect assignments of the ^{13}C NMR shifts.

A set of ^{13}C NMR data for **3** was also published by Fomácek and Kubeczka [10] for analysis of essential oils by GC and ^{13}C NMR spectroscopy. Nevertheless, these data (Table 1) were still nearly identical to those for **1** [2]. Compounds **2** and **1** are C-1 epimers, and their ^{13}C NMR data are clearly distinguishable (Table 1). Since (+)-ledol (mirror image of **1**) and (+)-viridiflorol (**3**) are also epimers with configurations different at C-10, one would expect two distinct sets of ^{13}C NMR data for each compound.

In order to confirm the identity of ledol isolated from the liverwort *C. recurvifolia* and that from Taiyo Koryo Co., a commercial sample of ledol was also obtained from the same company. It turned out that all spectral data were identical to those of our natural ledol. On inspection of the stereostructures of **1**, **2** and **3**, one would find that H-1 and H-14 (C_{10}-Me) are *cis* to each other in **1**, while *trans* in **2** and **3**. In other words, we may expect to observe some NOE effect between H-1 and H-14 in **1**, but impossible in **2** and **3**. In both NOESY and different NOE spectra of our commercial ledol, we indeed observed such correlations, which validated the identity of the commercial sample.

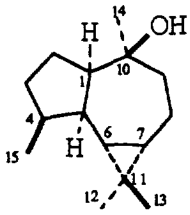
As shown in Table 1, although the proton chemical shifts of the four methyls of **1**, **2** and diastereomer A [6] are quite close, those of the two protons on the cyclopropyl ring, i.e. H-6 and H-7, are particularly distinguishable. Data for diastereomer A were extracted from refs [6, 7], in which the compound was claimed to be ledol, but the NMR data were different from those for ledol [2] as above mentioned.

From what is reported in the literature and the data from our own commercial samples, the values of $[\alpha]_D$ for both (+)-ledol and (+)-viridiflorol [5, 11] are rather small (2–10°), while that for (±)-globulol is relatively large (35–60°) [2, 5]. Since the $[\alpha]_D$ of our ledol from *C. recurvifolia* is −3.7°, and all aromadendranes isolated so far form liverworts belong to the *ent*-series, i.e. with an α-cyclopropyl ring [12], (−)-ledol with a stereostructure shown as **1** was thus found to be the first natural occurrence from plants. It so happened that (−)-ledol was also isolated from another Taiwanese liverwort *Bazzania tridens* [13], and their spectra were completely identical in every aspect. Compound **2** had been reported as a component from two liverwort species [1, 14]. Compound **3** has often been referred to as a component from various essential oils analysed by GC or GC–mass spectrometry, but its NMR data reported in the literature [8, 10] seem to be unreliable.

Other identified sesquiterpenes in the oil of *C. recurvifolia* by GC–mass spectrometry are bicycloelemene, *trans*-β-farnesene, bicyclogermacrene, spathulenol (**7**), 3α-acetoxybicyclogermacrene (**4**) and albicanol (**8**). No other species in the family of Cephaloziellaceae has been investigated chemically. The present study indicated that oxygenated sesquiterpenes were the major constituents for *C. recurvifolia*. No trace diterpenes (except phytol and phytadienes from chlorophyll) and aromatics were observed in the oil.

EXPERIMENTAL

General. Solvents used for spectral measurements were: $CDCl_3$ (^{1}H and ^{13}C NMR, 300, 500, 75 MHz); $CHCl_3$ ($[\alpha]_D$. GC-MS: 70 ev, column, DBWAX, 30 m × 0.25 mm, 50–220° (40 min), 5° min^{-1}.



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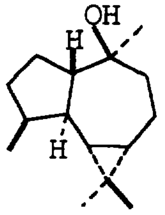

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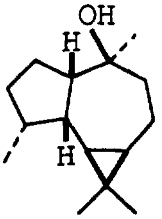

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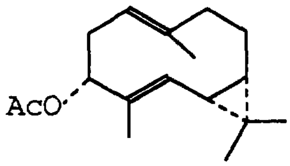

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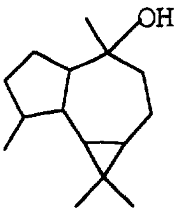

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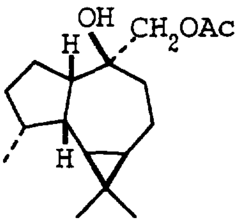

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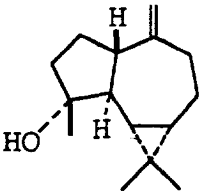

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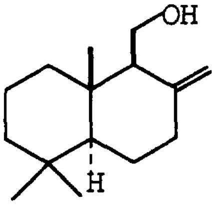

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Plant material. *Cephaloziella recurvifolia* Hatt. was collected in October 1993 at Wuyi Shan (*ca* 1200 m), Jianxi Province, China, and identified by Dr. K. Yamada (Ise-shi, Japan) and Mr C.-L. Liu (National Natural Science Museum of Shanghai). A voucher specimen was deposited at the Department of Chemistry, Tamkang University, Taiwan.

Extraction and isolation. Air-dried and powdered whole plant (70g) of *C. recurvifolia* was extracted with EtOAc. The crude extract (0.65 g) was chromatographed on silica gel using an *n*-hexane-EtOAc gradient for fractionating. Compound **3** (20 mg) was isolated from the 15% EtOAc–*n*–hexane eluate.

(−)-*Ledol* (**3**). $[\alpha]_D$ −3.7° (*c* 0.035); IR ν_{max}^{KBr} cm^{-1}: 3455; GC-MS(EI) *m*/*z* (rel. int.): 222 ($[M]^+$, 5), 189 (52), 161 (95), 122 (53), 121 (55), 109 (100), 107 (65), 105 (65), 93 (57), 81 (52), 69 (70), 43 (62).

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