

compound ($[\alpha]_D^{20}$, co-TLC, IR, NMR). An inseparable mixture of the two C-6 epimers **5** was also isolated.

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BICYCLOGERMACRENE TYPE SESQUITERPENOID FROM THE LIVERWORT *CONOCEPHALUM CONICUM*

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Key Word Index—*Conocephalum conicum*; Conocephalaceae; Hepaticae; bicyclogermacren-13-al; bicyclogermacrene; sesquiterpene aldehyde; bicyclo[8.1.0]undecane.

Abstract—From the thalloid liverwort *Conocephalum conicum*, a new sesquiterpene aldehyde has been isolated and its structure has been elucidated as bicyclogermacren-13-al by extensive 2D NMR spectroscopy.

INTRODUCTION

Two chemical studies on the thalloid liverwort *Conocephalum conicum* have been documented in the past decade [1]. The liverworts, however, can be regarded as one of the plant sources to search for biologically interested substances, as they produce a number of terpenoids [2, 3] and aromatic compounds [3], and also large amounts of material are readily available. As part of our program [4], we have re-examined *Conocephalum conicum* from Japan and have isolated a new sesquiterpene aldehyde (**1**) named bicyclogermacren-13-al. This paper describes the structure of the new sesquiterpene.

RESULTS AND DISCUSSION

A combination of column chromatography on silica gel, Sephadex LH-20, and HPLC of an ether extract of *C. conicum* led to the isolation of a new labile sesquiterpene (**1**) as a colourless oil.

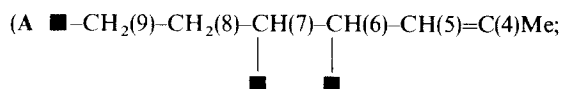
Compound **1** had the molecular formula $C_{15}H_{22}O$ (M^+ at m/z 218.1650) indicating five degrees of unsaturation. The IR, 1H NMR and ^{13}C NMR spectra (Table 1) indicated the presence of a formyl group (1690 cm^{-1} ; $\delta 9.60\text{ s}$). One tertiary methyl group ($\delta 1.27\text{ s}$), two olefinic methyl groups ($\delta 1.50$ and 1.69 , each s) which were long range coupled with the olefinic protons at $\delta 4.96$ (2 H, m) on two trisubstituted double bonds ($\delta 122.2$ and 125.8 , each d , 131.3 and 139.8 , each s). These spectral data showed that **1** was a bicyclosesesquiterpene. The 2D 1H – 1H and ^{13}C – 1H COSYs were extensively examined to clarify the connectivity of each proton in **1**. One of the two olefinic proton signals ($\delta 122.2$ and 125.8) eventually appeared at the same field ($\delta 4.96$) and displayed a coupling to the signals at $\delta 2.13$ (dd , $J = 11.7, 8.8\text{ Hz}$), which were further coupled with the high field signals at $\delta 1.43$ (ddd , $J = 12.5, 8.8, 2.9\text{ Hz}$). Moreover, this methine signals had cross peaks with the two geminal proton signals ($\delta 1.94$ and 2.05 ; $\delta 25.8$), each of which showed additional cross peaks with the methylene signals ($\delta 1.75$ and 2.52 ;

Table 1. ^1H and ^{13}C NMR spectral data of compound **1***

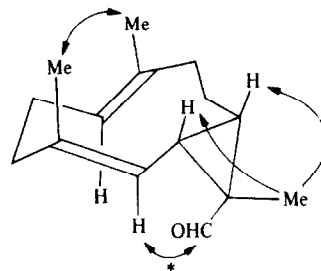
Carbon no.	C	H
1	125.8	4.96 (m)
2	26.0	1.98 (m), 2.10 (m)
3	40.9	1.96 (m) 2.26 (ddd, 11.7, 3.7, 3.7)
4	131.3	
5	122.2	4.96 (m)
6	35.2	2.13 (dd, 11.7, 8.8)
7	36.9	1.43 (ddd, 12.5, 8.8, 2.9)
8	25.8	1.94 (dddd, 13.2, 13.2, 12.5, 3.5) 2.05 (dddd, 13.2, 4.4, 3.5, 2.9)
9	37.0	1.75 (ddd, 13.2, 13.2, 4.4) 2.52 (ddd, 13.2, 3.5, 3.5)
10	139.8	
11	35.7	
12	20.1	1.27 (s)
13	203.5	9.60 (s)
14	16.2	1.69 (br s)
15	20.8	1.50 (br s)

*All assignments were confirmed by the ^1H - ^1H , ^{13}C - ^1H , and long-range ^{13}C - ^1H COSY spectra.

$\delta 37.0$). On the other hand, the other olefinic proton signals revealed cross peaks with the poor resolved geminal proton signals ($\delta 1.98$ and 2.10 ; $\delta 26.0$), which were coupled with other geminal proton signals at $\delta 1.96$ (m) and 2.26 (ddd, $J = 11.7, 3.7, 3.7$ Hz). These results led to two partial segments:

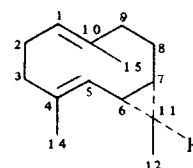


(B) \blacksquare -CH₂(3)-CH₂(2)-CH(1)=C(10)Me. It was further possible to correlate each segment by the analyses of the long range ^{13}C - ^1H COSY of **1**. The highest field quaternary carbon signal at $\delta 35.7$ showed long range couplings to both the formyl and methyl proton signals. This methyl signal was found to be further correlated to the C-6 and C-7 carbon signals indicating that the partial structure (A) contained a cyclopropane ring with methyl and formyl substituents bonded at C-6 and C-7, respectively. In addition, the olefinic methyl signals at $\delta 1.50$ (15-Me) and 1.69 (14-Me) revealed clear cross peaks with the quaternary carbon signals at $\delta 139.8$ (C-10) and 131.3 (C-4), as well as with the C-9 carbon signal ($\delta 37.0$) in A and the C-3 carbon signal ($\delta 40.9$) in B, respectively. Consequently, the partial structures (A) and (B) must be connected through C-9-C-10 and C-3-C-4. The above spectral evidence disclosed that **1** comprised a bicyclo[8.1.0]undecane skeleton. The geometries of both the trisubstituted double bonds were determined to be *trans* (*E*-form) not only from the diagnostic shifts of the olefinic methyl carbon (16.2 and 20.8) [4] but also from no detection of NOEs between the methyl groups and the olefinic methyl carbon (16.2 and 20.8) [5] but also from correspond to bicyclogermacrene-13-al or 12-al. The NOE difference spectra of **1** shown in Fig. 1 suggested that **1** adopts the conformation with a cross orientation of the



* The observed NOEs of **1** are indicated by arrows

Fig. 1.



1 R = CHO

2 R = Me

trans-double bonds as reported by Tori *et al.* [6, 7] and thereby it was evident that the C-13 methyl group in **2** was oxidized into an aldehyde group. Accordingly, the structure of **1** was elucidated as bicyclogermacrene-13-al.

Its absolute stereochemistry is assumed to be the *ent*-type in the light of the fact that bicyclogermacrene (**2**) and all other sesquiterpenes so far isolated from *C. conicum* are enantiomeric to those which occur in higher plants [3]. However, our efforts to convert **1** into the known bicyclogermacrene (**2**) were unsuccessful due to an extreme instability of **1** under reductive conditions.

EXPERIMENTAL

^1H NMR (400 MHz) and ^{13}C NMR (100.16 MHz): CDCl_3 , TMS as int. standard; CC: silica gel (Merck, 230-400 mesh); TLC: precoated silica gel (Merck, 0.25 mm). Spots were visualized by 30% H_2SO_4 and 2,4-DNP followed by heating.

Plant material. The liverwort, *Conocephalum conicum* (L.) Lindb. was collected in southern Tokushima on May 31, 1987 and identified by Y.A. A voucher specimen has been deposited at the Herbarium of Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. Air-dried and powdered plants were extracted with Et_2O at room temp. for one month. The Et_2O extract was evapd *in vacuo* to give a crude fragrant extract (7.45 g), which was divided into nine fractions by CC on silica gel using a *n*-hexane-EtOAc gradient. The fraction (1.3 g) eluted with *n*-hexane-EtOAc (1:9) was rechromatographed on Sephadex LH-20 (CHCl_3 -MeOH, 1:1) and finally purified by HPLC [column: Lichrop Si 60 Type A, Sol: *n*-hexane-EtOAc (1:19), psi: 15 kg/cm²] to yield bicyclogermacrene-13-al (**1**) (21.7 mg) as a colourless oil: $[\alpha]_D^{20} = -52.0$ (CHCl_3 ; c 0.4); UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm: 218 (ϵ 11300); IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 2950, 2850, 1690 (C=O); MS (rel. int.) 218.1650 [$\text{M}]^+$ (28) (calc. 218.1671 for $\text{C}_{15}\text{H}_{22}\text{O}$), 161 (45), 145 (43), 133 (51), 121 (81), 105 (81), 93 (100), 91 (93), 81 (71), 79 (76), 67 (53); ^1H NMR and ^{13}C NMR: see Table 1.

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MANDASSIDIONE AND OTHER SESQUITERPENIC KETONES FROM *CYPERUS ARTICULATUS*

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Key Word Index—*Cyperus articulatus*; Cyperaceae; sesquiterpenes; mandassidione; mustakone; isopatchoulene.

Abstract—A new monocyclic sesquiterpenic diketone, mandassidione, was isolated from the rhizomes of *Cyperus articulatus*, along with mustakone and isopatchoul-4(5) en-3-one. The structures were established from spectroscopic and chemical evidence.

INTRODUCTION

Cyperus articulatus known in Cameroon as 'mandassi' is a tropical sedge widely used in traditional medicine. It is also used as perfume by traditional healers. Earlier studies on the essential oils from *Cyperus articulatus* led to the isolation of a bicyclic ketone named articulone, the structure of which was later revised and renamed cyperone, then finally isopatchoul-4(5) en-3-one, **1** [1–5]. Recently we described the isolation and structural elucidation of corymbolone and α -corymbolol from the *n*-hexane extract of *C. articulatus* [6]. We now report on the structural determination of a new compound, mandassidione **2**, isolated from the same extract together with known isopatchoulene **1** and mustakone **3**.

RESULTS AND DISCUSSIONS

The *n*-hexane extract of *C. articulatus* rhizomes was fractionated by column chromatography on silica gel with *n*-hexane–ethyl acetate mixture (EtOAc) of increasing polarity. Part of fraction eluted with (hexane–EtOAc: 19:1) was further purified by CC and preparative TLC to give mustakone **3** as a colourless oil ($[\alpha]_D^{25}$ –42.5°). The remaining part was converted into 2,4-dinitrophenylhydrazone derivatives from which two 2,4-DNP hydrazone derivatives were further separated by fractional crystallization. The first was determined as 2,4-DNP hydrazone of

mustakone (**4**) from a spectroscopic study, while the second was identified as 2,4-DNP hydrazone of isopatchoulene (**5**) by comparison of its physical and spectral data with that already published [2–5].

As the structure of mustakone was previously assigned mainly by chemical means [7, 8] its spectral data will be briefly described here. We observed the molecular ion of mustakone in its EI mass spectrum at m/z 218, in agreement with the molecular formula $C_{15}H_{22}O$. The IR spectrum was indicative of an α,β -unsaturated ketone (ν 1673 cm^{-1}). The ^{13}C NMR spectrum showed the signals of the 15 carbon atoms among which that of the carbonyl group (δ 203.47) and those of the double bond (δ 121.39 and 169.5 s). Assignments of the 1H NMR spectrum were made by using 2D COSY and led to the structure **3** of mustakone. We observed the large value (6.7 Hz) of the $^4J_{HH}$ coupling constant between H-1 and H-5, characteristic of *cis* protons located on opposite vertices of a cyclobutane ring [9].

The fraction eluted with mixture (hexane–EtOAc: 9:1) yielded corymbolone, while that of eluent mixture (hexane–EtOAc 4:1) yielded a mixture of α -corymbolol and mandassidione which were further separated by CC.

Mandassidione **2**, ($[\alpha]_D^{25}$ –9.1°) was obtained as a colourless oil and analysed for $C_{15}H_{22}O_2$ from mass spectral data. This molecular formula implied five unsaturated sites in a monocyclic structure since the