

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 bicyclosesquiterpene. 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)

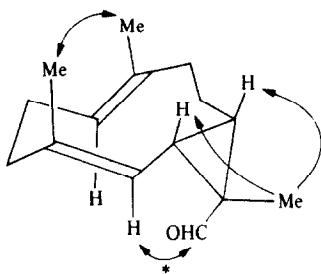
*All assignments were confirmed by the ^1H - ^1H , ^{13}C - ^1H , and long-range ^{13}C - ^1H COSY spectra.

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

(A) ■-CH₂(9)-CH₂(8)-CH(7)-CH(6)-CH(5)=C(4)Me;

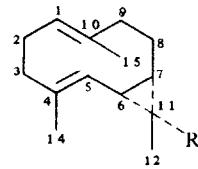


(B) ■-CH₂(3)-CH₂(2)-CH(1)=C(10)Me. It was further possible to correlate each segment by the analyses of the long range ^{13}C - ^1H COSY of **1**. The highest field quaternary carbon signal at δ 35.7 showed long range couplings to both the formyl and methyl proton signals. This methyl signal was found 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 δ 1.50 (15-Me) and 1.69 (14-Me) revealed clear cross peaks with the quaternary carbon signals at δ 139.8 (C-10) and 131.3 (C-4), as well as with the C-9 carbon signal (δ 37.0) in **A** and the C-3 carbon signal (δ 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 bicyclogermacen-13-al (**1**) (21.7 mg) as a colourless oil: $[\alpha]_{\text{D}}^{20} = -52.0$ (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 [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; isopatchoulenone.

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 cypernone, 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 isopatchoulenone **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 ($[\alpha]_D^{25} -42.5^\circ$). 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 isopatchoulenone (**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 ($\nu 1673\text{ cm}^{-1}$). The ^{13}C NMR spectrum showed the signals of the 15 carbon atoms among which that of the carbonyl group ($\delta 203.47$) and those of the double bond ($\delta 121.39$ and 169.5 s). Assignments of the ^1H NMR spectrum were made by using 2DCOSY 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**, ($[\alpha]_D^{25} -9.1^\circ$) 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