

MONO- AND SESQUITERPENOIDS OF *CONOCEPHALUM SUPRADECOMPOSITUM*

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Key Word Index—*Conocephalum supradecompositum*; Conocephalaceae; Marchantiales; Hepaticae; mono- and sesquiterpenoids; germacranolides; (+)-costunolide; (+)-dihydrocostunolide; plant growth inhibitory activity; chemosystematics.

Abstract—The liverwort *Conocephalum supradecompositum* belonging to the Marchantiales is a rich source of the germacranolides (+)-costunolide and (+)-dihydrocostunolide. *C. supradecompositum* is chemically very different from *C. conicum* and *Wiesnerella denudata* and it may be more primitive than the latter two species. The plant growth inhibitory activity of *C. supradecompositum* is due to (+)-costunolide.

INTRODUCTION

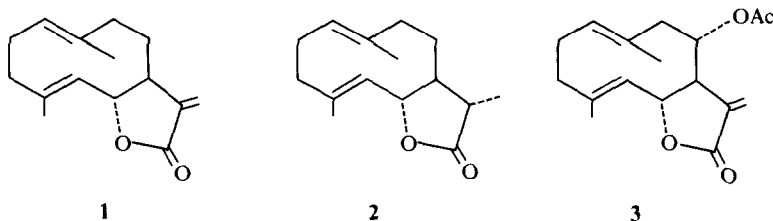
Most liverworts belonging to the Jungermanniales produce mono-, sesqui- and/or diterpenoids as major components. Some species belonging to Marchantiales also elaborate much terpenoid. In Conocephalaceae (Marchantiales), two genera, *Conocephalum* and *Wiesnerella*, are known, and *C. conicum* (L.) Dum. and *C. supradecompositum* (Lindb.) Steph. (= *Sandea supradecomposita*) in the former genus and *W. denudata* (Mitt.) Steph. in the latter genus are present in Japan. In comparison with *C. conicum* and *W. denudata*, *C. supradecompositum* is small and its thallus is 1–3 cm × 2–3 cm. Recently, we reported that *C. conicum* and *W. denudata* elaborated a large amount of monoterpene hydrocarbons, monoterpene acetate, and sesquiterpene lactones which inhibited the germination and root elongation of rice in husk, and that the former species was chemically close to the latter one [1, 2]. The plant growth inhibitory activity is also observed in the crude extract of *C. supradecompositum* which proved to be a rich source of germacranolides. The present communication is concerned with the distribution of mono- and sesquiterpenoids in *C. supradecompositum*.

RESULTS AND DISCUSSION

Air-dried and ground female and male gametophytes and the sterile gametophytes collected in four different locations were extracted with Et₂O. Each crude extract was first analysed by GC/MS. The GC and TLC showed

that the chemical constitutions of the female, male and sterile gametophytes were identical and the crude extracts were combined and then directly chromatographed on Si gel to afford two sesquiterpene lactones (**1** and **2**) as the major components. The stereostructures of the germacranolides (**1** and **2**) deduced from the spectral data (¹H NMR, IR, MS and CD) were confirmed by identity with the spectral data of the authentic (+)-costunolide and (+)-dihydrocostunolide. In addition to the above costunolides, large amounts of campesterol, stigmastanol and sitosterol were obtained. Furthermore, β-pinene, myrcene, limonene, β-elemene, β-caryophyllene, ar-curcumen and cuparene have been detected by GC/MS as very minor components [3, 4].

C. supradecompositum is chemically very different from *C. conicum*. The latter species thus elaborates a large amount of monoterpene hydrocarbons and bornyl acetate, and the chemical constituents of the female and male gametophytes are different, particularly in sesquiterpene lactones. On the other hand, the female and male gametophytes of *C. supradecompositum* elaborate the same components whose content is almost identical. In *C. supradecompositum*, tulipinolide (**3**) and its related guaianolides which have been isolated from *C. conicum* and *W. denudata* have not been detected and (+)-costunolide (**1**) and (+)-dihydrocostunolide (**2**), which are the precursors of tulipinolide and its derivatives, have been elaborated as major components. Furthermore, the monoterpene content in *C. supradecompositum* is considerably less than that of *C. conicum* and their



chemical constitutions are also quite different. It is interesting from the evolutionary viewpoint of Conocephalaceae that *C. supradecompositum* lacks the capacity to introduce the 8 α -acetoxyl group into (+)-costunolide (**1**) and to elaborate further the related guaianolides.

On the basis of the above results, it is suggested that *C. supradecompositum* is more primitive than *C. conicum* and *W. demodata*. Lindberg [5] proposed the name *Sandea supradecomposita* Lindb. for *C. supradecompositum*. The name *Sandea* was also revived as a subgenus of *Conocephalum* by Inoue [6]. On the basis of the above chemical evidence, we think that *Sandea* should be re-adopted as a monotypic genus.

The crude extract of *C. supradecompositum* showed inhibitory activity against the germination and root elongation of rice in husk at ca 1000 ppm. The active substance is (+)-costunolide (**1**) which showed the above activity at ca. 200 ppm.

EXPERIMENTAL

The solvents used for spectral determination were: TMS-CDCl₃ (¹H NMR); MeOH (CD); CHCl₃ (IR). [α]_D values were measured in MeOH. TLC: precoated Si gel (0.25 mesh) F₂₅₄, solvent system: *n*-hexane-EtOAc (4:1), C₆H₆-EtOAc (4:1). Spots were detected by UV light (254 nm) and spraying with 30% H₂SO₄ and then heating at 100°. GC/MS: 70 eV, column, 1% SE-30, 2 mm \times 3 m, temp. programme 50–270° at 5°/min, He 30 ml/min. GC: column, 5% SE-30, 2 mm \times 3 m, temp. programme 50–270° at 5°/min, N₂ 30 ml/min.

Plant materials. *Conocephalum supradecompositum* (Lindb.) Steph. (= *Sandea supradecomposita*), identified by Dr. S. Hattori, is deposited in the Herbarium, Institute of Pharmacognosy, Tokushima Bunri University.

Bioassay with rice in husk. The bioassay of the crude extract of *C. supradecompositum* and (+)-costunolide (**1**) was carried out by the method of Kato *et al.* [7].

Extraction and isolation. *Conocephalum supradecompositum* was collected in the different locations and then air-dried for 5 days. Mie prefecture in July 1978 (male gametophyte, 20.20 g), Yada-cho, Tokushima prefecture in April 1979 (female gametophyte, 6.50 g and male one, 16.00 g), Tottori prefecture in October 1979 sterile gametophyte, 126.05 g) and Anan-shi,

Tokushima prefecture in April 1980 (sterile gametophyte, 11.90 g). Each sample was ground and extracted with Et₂O for 10 days. The crude extracts were monitored by TLC and GC. The chromatograms of the crude extracts were identical and then they were combined. The total extract (2.720 g) was directly chromatographed on Si gel using *n*-hexane-EtOAc gradient and divided into 7 fractions. The first fraction eluted with *n*-hexane contained mono- and sesquiterpene hydrocarbons (9 mg) in which β -pinene, myrcene, limonene, β -elemene, β -caryophyllene, ar-curcumen and cuparene have been detected by GC/MS [3, 4]. The second fraction (*n*-hexane-EtOAc, 19:1) gave a very fragrant oil (32 mg), not identified. The third fraction (9:1) gave triglycerides (670 mg). The fourth fraction (4:1) contained sesquiterpene lactone mixtures (730 mg) which were rechromatographed on Si gel using a C₆H₆-EtOAc gradient to afford (+)-costunolide (**1**) (503 mg) and (+)-dihydrocostunolide (**2**) (175 mg) whose spectral data, and mps and [α]_D were identical to those of the authentic samples. The fifth fraction (3:1) gave a dark green oil (420 mg) which was rechromatographed on Si gel to afford phytosterols (345 mg) (campesterol: stigmastanol: sitosterol = 1:1:2 in GC/MS). The sixth fraction (3:2) gave fatty acids (101 mg). From the seventh fraction, unidentified alcohols (157 mg) were obtained.

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