

¹³C NMR CHEMICAL SHIFTS OF OVALIFOLIENE AND RELATED COMPOUNDS WITH THE 2,3-SECO-ALLOAROMADENDRANE SKELETON: STRUCTURE OF (+)-9 α -ACETOXYOVALIFOLIENE, A PLANT GROWTH INHIBITOR

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Key Word Index—*Plagiochila semidecurrans*; Plagiochilaceae; Bryophyta; ¹³C NMR; plant growth-inhibitors; sesquiterpenoids; 2,3-*seco*-alloaromadendrane; acetyl hemiacetals; ovalifoliene; ovalifolienal; ovalifolienalone; 9 α -acetoxyovalifoliene; plagiochiline A and B.

Abstract—¹³C NMR spectra of several 2,3-*seco*-alloaromadendrane-type acetyl hemiacetals were analysed and the structure of an additional plant growth-inhibitor was determined to be (+)-9 α -acetoxyovalifoliene. The biological activity of the new compound is also described.

INTRODUCTION

Liverworts (Hepaticae) contain characteristic oil bodies in each cell of the gametophytes which grow from the spores and form the usual plant bodies. From these plants, sesquiterpenoids with a number of novel carbon skeletons have been isolated [1-6]. The liverwort sesquiterpenoids are normally the enantiomeric forms of the corresponding products of higher plants as those of fungi and marine invertebrates [7]. In previous papers, we reported the isolation from the liverworts *Plagiochila semidecurrans* and *P. ovalifolia*, of three new sesquiterpenoids, (+)-ovalifoliene (1),† (+)-ovalifolienal (3) and (+)-ovalifolienalone (3), which have remarkable plant growth-inhibiting effects. The structures and absolute configurations of these compounds were determined as enantiomeric acetyl hemiacetal sesquiterpenoids with a novel 2,3-*seco*-alloaromadendrane carbon skeleton [8-10]. Continuous screening for the plant growth inhibitors was performed on the genus *Plagiochila* and has resulted in the isolation from *P. semidecurrans* of an additional new acetyl hemiacetal, (+)-9 α -acetoxyovalifoliene (5) with the same carbon skeleton as the above biologically active acetyl hemiacetals. In addition, (+)-plagiochiline A (6) and (+)-plagiochiline B (7) have been isolated. These are compounds which have been obtained previously from *P. yokogurensis* and *P. hattoriana*§ [12, 13].

RESULTS AND DISCUSSION

Analysis of ¹³C NMR spectra of ovalifoliene (1), ovalifolienal (2), ovalifolienalone (3) and 10(15)-epoxyovalifoliene (4)

¹³C NMR spectra of compounds 1-4 were analysed by the single frequency off-resonance decoupling technique, application of the known chemical shift rules [14-16] and comparison of the spectra of the examined compounds. The correlation results thus obtained are shown in Table 1. In these compounds the chemical shifts of C-1, C-2, C-3, C-4 and C-5, of the hemiacetal ring, showed a good correspondence to each other and agreed with those of the corresponding carbons in irridoids [17, 18]. The chemical shifts of C-1, C-5, C-6, C-7, C-8, C-9, C-10 and C-11, components of the substituted bicyclo[5.1.0]octane system, were comparable with those of *seco*-aromadendrane-type sesquiterpenoids, [(2, 3] and A. Matsuo *et al.*, unpublished data for myliol and 3-acetoxytaylorione) guaianes-type sesquiterpenoids [19] and guaianes-type diterpenoids [20].

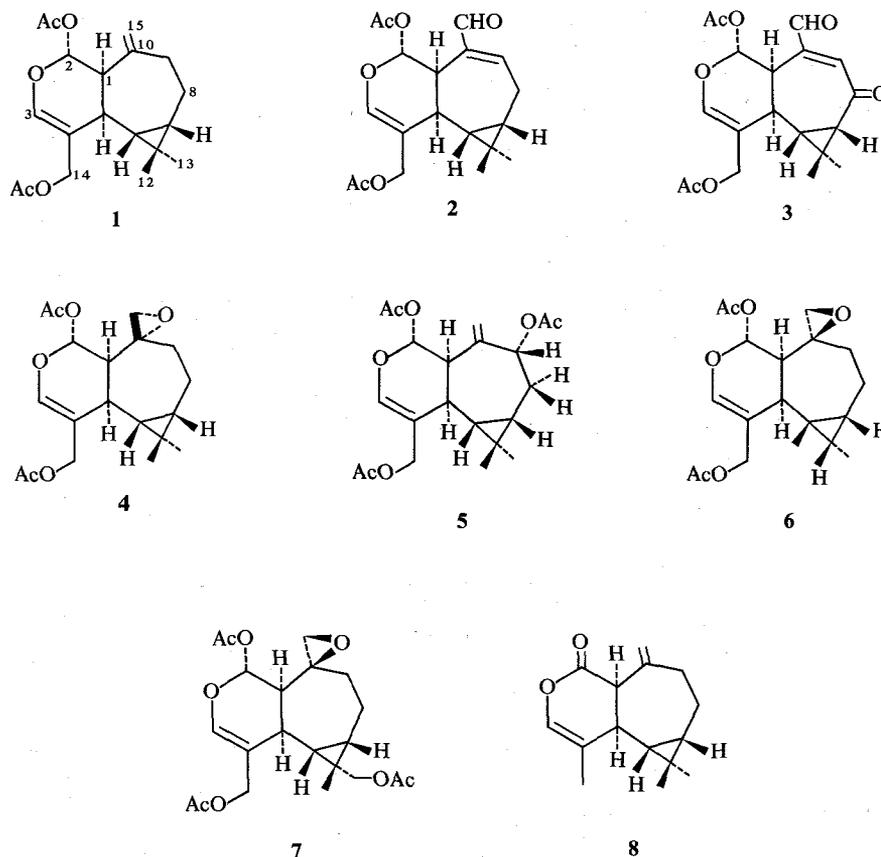
Structure of (+)-9 α -acetoxyovalifoliene (5)

The IR and ¹H NMR spectra were similar to those of the *seco*-alloaromadendrane-type acetyl hemiacetals (1-3). The spectra also suggested that it was a tricyclic sesquiterpenoid containing, together with an additional secondary acetoxy group, the following common groups; an acetyl hemiacetal, β -substituted vinyl ether, a primary acetoxy group on the vinyl carbon, a *gem*-dimethyl-substituted cyclopropane ring with two methines and an exomethylene group.

When the centre of the proton signal (δ 5.02, *dd*, $J_{9-\alpha} = 9.0$ and $J_{9-\beta} = 3.0$) assigned to the methine bearing the secondary acetoxy group was irradiated, both the signals at δ 1.32 and 2.33 assigned as an axial proton and an equatorial proton on C-8 respectively, were deformed. Irradiation at δ 2.33 (H-8 β) resulted in loss of

†Recently, Asakawa *et al.* [11] reported the isolation from the same liverwort of the acetyl hemiacetal plagiochiline C, which may be the same compound as (+)-ovalifoliene, together with three related acetyl hemiacetals.

§The stereochemistry of C-10 and C-11 in the compounds was not confirmed in the literature, but the configurations of both carbons were tentatively proposed by considering the biogenesis of the compounds [11].



the coupling of 3.0 Hz reforming the double doublet at δ 5.02 into a doublet ($J_{9-8\alpha} = 9.0$), and irradiation at 1.32 (H-8 α) reformed the double doublet into a doublet ($J_{9-8\beta} = 3.0$). Such decoupling results revealed that the secondary acetoxy group was on the allylic carbon (C-9) with the α -configuration (equatorial) because the adjacent protons were the methylene protons at δ 1.32 and 2.33, and the coupling constants ($J_{9-8\alpha} = 9.0$ and $J_{9-8\beta} = 3.0$) were explained as only *a-a* and *a-e* types.

The above structure of the new plant growth inhibitor was supported by the ^{13}C off-resonance NMR spectrum showing 6 *s*, 7 *d*, 3 *t* and 5 *q* (Table 1). This fitted well with the spectrum of ovalifoliene (1) except for one triplet at δ 31.8 and one doublet at δ 73.7 attributed to C-8 and C-9, respectively. Although both the exceptional signals were shifted by 6.2 and 38.3 ppm to a lower field than those of the corresponding carbons in ovalifoliene, the shifts could be reasonably explained by β - and α -effects of the acetoxy group. Thus, the structure of the compound was determined to be 9 α -acetoxyovalifoliene (5).

^{13}C NMR spectra of plagiochiline A (6), plagiochiline B (7) and plagiochilide (8)

In connection with the spectroscopic investigation of the acetyl hemiacetals, ^{13}C NMR spectra of plagiochiline A (6), B (7) and plagiochilide (8) were analysed (Table 1). In the spectra of both plagiochilines (6, 7), the signals of C-10 and C-15 bearing the epoxy ring appeared at δ 60.1 and 51.7 and δ 60.0 and 51.6, respectively. These signals were shifted to lower field by *ca* 0.7 ppm and higher field by *ca*

5.7 ppm, by comparison with those of the corresponding carbons in the synthesized epoxyovalifoliene (4). Such shifts of both the signals indicated that in plagiochilines the epoxy ring was not arranged in the same α -configuration as that of 10(15)-epoxyovalifoliene but in the reversed β -configuration [8]. Recently, the ^{13}C NMR spectrum of plagiochilide (8) and its assignment has been reported [21]. Although the assignment should be interchanged between C-12 and C-14, all of the signals showed a good agreement with those of the above *seco*-alloaromadendrane-type hemiacetals (1-7).

Regarding the spectrum of plagiochiline B (7), a quartet at δ 24.3 was assigned to C-12 and a triplet at δ 65.4 to C-13. This was reasonable considering that the signal for C-13 was shifted from δ 15.8 and that for C-12 from δ 29.8 in the spectrum of plagiochiline A (6) owing to α - and γ -effects of the primary acetoxy group. Thus the acetoxy methyl group must be attached to the cyclopropane ring with the α -configuration, that is, the stereochemistry of C-11 was determined as *S*.

Biological activity of 9 α -acetoxyovalifoliene (5)

The plant growth-inhibiting activity of the new acetyl hemiacetal (5) was tested on rice seedlings. The inhibition of the growth of leaves and roots was almost complete at a concentration of 25 ppm, and the concentration for 50% growth-inhibition was about 12 ppm. It is considered that this strong inhibitor competing with the activity of compounds 1, 2 and 4, together with the other sesquiterpene acetyl hemiacetals, may act as one of the important allomones in the ecological system of the plant.

Table 1. ¹³C NMR chemical shifts of several acetyl hemiacetals with the 2,3-*seco*-alloaromadendrane skeleton

	1	2	3	4	5	6	7	8*
Carbon No.								
1	51.7 <i>d</i>	32.1 <i>d</i>	39.1 <i>d</i>	50.7 <i>d</i>	48.4 <i>d</i>	50.0 <i>d</i>	50.1 <i>d</i>	53.7 <i>d</i>
2	91.5 <i>d</i>	92.5 <i>d</i>	92.2 <i>d</i>	91.7 <i>d</i>	90.3 <i>d</i>	92.0 <i>d</i>	92.0 <i>d</i>	170.0 <i>s</i>
3	140.4 <i>d</i>	141.4 <i>d</i>	141.6 <i>d</i>	140.3 <i>d</i>	140.5 <i>d</i>	140.5 <i>d</i>	140.9 <i>d</i>	135.1 <i>d</i>
4	116.3 <i>s</i>	115.5 <i>s</i>	116.0 <i>s</i>	116.5 <i>s</i>	113.3 <i>s</i>	116.2 <i>s</i>	116.0 <i>s</i>	124.5 <i>s</i>
5	34.3 <i>d</i>	30.8 <i>d</i>	37.9 <i>d</i>	31.4 <i>d</i>	33.7 <i>d</i>	31.8 <i>d</i>	31.6 <i>d</i>	38.8 <i>d</i>
6	29.6 <i>d</i> †	28.4 <i>d</i>	35.4 <i>d</i>	29.6 <i>d</i>	29.7 <i>d</i>	28.7 <i>d</i>	30.5 <i>d</i>	29.3 <i>d</i>
7	29.4 <i>d</i> †	24.4 <i>d</i>	32.3 <i>d</i>	28.8 <i>d</i>	28.5 <i>d</i>	27.8 <i>d</i>	27.8 <i>d</i>	27.6 <i>d</i>
8	25.6 <i>t</i>	39.8 <i>t</i>	198.8 <i>s</i>	21.0 <i>t</i>	31.8 <i>t</i>	21.9 <i>t</i>	22.1 <i>t</i>	25.4 <i>t</i>
9	35.4 <i>t</i>	154.4 <i>d</i>	146.5 <i>d</i>	33.7 <i>t</i>	73.7 <i>d</i>	33.9 <i>t</i>	33.9 <i>t</i>	35.2 <i>t</i>
10	148.7 <i>s</i>	140.1 <i>s</i>	145.2 <i>s</i>	59.3 <i>s</i>	146.8 <i>s</i>	60.1 <i>s</i>	60.0 <i>s</i>	147.4 <i>s</i>
11	19.7 <i>s</i>	21.0 <i>s</i>	29.6 <i>s</i>	20.3 <i>s</i>	19.7 <i>s</i>	19.1 <i>s</i>	21.7 <i>s</i>	19.6 <i>s</i>
12	28.8 <i>q</i>	25.6 <i>q</i>	23.9 <i>q</i>	28.8 <i>q</i>	24.0 <i>q</i>	29.8 <i>q</i>	24.3 <i>q</i>	28.7 <i>q</i> †
13	15.6 <i>q</i>	15.6 <i>q</i>	16.6 <i>q</i>	15.7 <i>q</i>	15.8 <i>q</i>	15.8 <i>q</i>	65.4 <i>t</i>	15.5 <i>q</i> †
14	63.5 <i>t</i>	63.3 <i>t</i>	63.4 <i>t</i>	63.3 <i>t</i>	63.5 <i>t</i>	63.4 <i>t</i>	63.5 <i>t</i>	16.2 <i>q</i> † †
15	116.4 <i>t</i>	194.1 <i>d</i>	193.5 <i>d</i>	57.4 <i>t</i>	115.0 <i>t</i>	51.7 <i>t</i>	51.6 <i>t</i>	116.9 <i>t</i>
	170.9 <i>s</i>	171.1 <i>s</i>	169.1 <i>s</i>	171.3 <i>s</i>	170.9 <i>s</i>	171.0 <i>s</i>	171.5 <i>s</i>	
O	169.7 <i>s</i>	169.9 <i>s</i>	160.6 <i>s</i>	169.7 <i>s</i>	170.0 <i>s</i>	170.1 <i>s</i>	170.9 <i>s</i>	
	21.0 <i>q</i>	21.0 <i>q</i>	21.0 <i>q</i>	21.0 <i>q</i>	170.0 <i>s</i>	21.2 <i>q</i>	169.9 <i>s</i>	
—C—Me	20.9 <i>q</i>	20.0 <i>q</i>	20.6 <i>q</i>	21.0 <i>q</i>	21.0 <i>q</i>	21.2 <i>q</i>	21.2 <i>q</i>	
					21.0 <i>q</i>		21.0 <i>q</i>	
					21.0 <i>q</i>		21.0 <i>q</i>	

* The chemical shifts are reproduced from ref. [21].

† The assignment may be interchanged.

‡ The assignment was contrary to the lit. [21], but it was interchanged based on comparison with the data of the other compounds (1–7).

EXPERIMENTAL

IR spectra were recorded on a grating spectrometer for soln in CCl₄ and mass spectra were determined at 70 eV. Optical rotations were taken on an automatic polarimeter for CHCl₃ soln at room temp. ¹H NMR spectra were measured at 60 MHz and 90 MHz, and ¹³C NMR at 22.63 MHz for CDCl₃ soln with o.d. 8 mm tubes using TMS as internal reference. The chemical shifts were given in ppm (δ) relative to the internal standard. Si gel 60 (No. 7734) and Si gel 60 PF₂₅₄ (No. 7747) were used for CC and TLC. Spots on TLC were visualized under a UV light or by spraying with 10% H₂SO₄ in EtOH followed by heating.

Material and its extraction. The liverwort *P. semidecurrans* was collected in a forest in Kochi-prefecture. The whole plant (1.3 kg), dried in the shade for several days, was digested with MeOH at room temp for 1 week. The solvent was removed *in vacuo*, and the brownish substance thus obtained was again extracted with Et₂O and the solvent was distilled to leave a dark greenish extract (41 g).

Isolation of (+)-9α-acetoxyovalifoliene (5), (+)-plagiochiline A (6) and (+)-plagiochiline B (7). The extract (40 g) was chromatographed through a Si gel column with hexane and EtOAc and divided into 12 fractions. (+)-9α-Acetoxyovalifoliene (5) (50 mg) was isolated from fraction 7 by CC (C₆H₆–Et₂O, 6:1) and preparative TLC (hexane–Et₂O, 2:1), yield 1.3% from the Et₂O extract. (+)-9α-Acetoxyovalifoliene (5): [α]_D²⁰ +53.2° (c 0.55); IR ν_{max}^{CCl₄} cm⁻¹: 1760, 1740, 1675, 1640, 1380, 1370, 1240, 915; ¹H NMR: δ 0.97 (3H, s), 1.07 (3H, s), 2.02 (6H, s), 2.09 (3H, s), 2.89 (1H, dd, J = 9.5 and 4.5 Hz), 4.33 (1H, d, J = 12.5 Hz), 4.51 (1H, d, J = 12.5 Hz), 4.92 (1H, s), 5.02 (1H, dd, J = 9.0 and 3.0 Hz), 5.07 (1H, s), 6.33 (1H, s), 6.51 (1H, d, J = 9.5 Hz); MS *m/e*

(rel. int.): 392 (M⁺, C₂₁H₂₈O₇, 3), 332.1649 (M⁺ – 60, C₁₉H₂₄O₅, calcd 332.1622, 33), 272.1394 (M⁺ – 120, C₁₇H₂₀O₃, calcd 272.1411, base), 230.1283 (M⁺ – 162, C₁₅H₁₆O₂, calcd 230.1305, 48), 215.1044 (M⁺ – 177, C₁₄H₁₅O₂, calcd 215.1070, 23), 212.1168 (M⁺ – 180, C₁₅H₁₆O, calcd. 212.1200, 72), 187 (45), 159 (36), 135 (27), 119 (22), 105 (28), 95 (30), 91 (33), 79 (27), 67 (18), 60.0214 (C₂H₄O₂, calcd 60.0211, 12), 57 (19), 55 (24). (+)-Plagiochiline A (6) (95 mg) and (+)-plagiochiline B (7) (45 mg) were isolated from Fr. 9 and Fr. 11 respectively, by CC (C₆H₆–Et₂O, 6:1 for 6 and 7) and prep. TLC (hexane–Me₂CO, 3:1 for 6; hexane–Et₂O, 1:2 for 7).

Growth-inhibitory activity on rice seedlings. The physiological activity of (+)-9α-acetoxyovalifoliene (5) was tested by using rice seedlings (Shin-sen-bon, an ordinary variety of *Oryza sativa* L.). The seeds were sterilized with EtOH and then with 1% NaClO soln and then germinated in water for 2 days at 30° under illumination. Groups of 7 seedlings all at the same level of germination were placed in flat-bottomed test tubes (2.5 × 13 cm) containing 2 ml of aq. soln at various concns of inhibitor (Tween 80 at 100 ppm was added to the aq. soln to dissolve the test compounds). After growing at 30° for 7 days under illumination, both the length of the second-leaf sheaths and the wt of roots were measured. The average length and total wt of 7 seedlings at each concn were as follows; 0 ppm: 25.8 mm and 107.6 mg, 1 ppm: 26.4 and 99.7, 5 ppm: 22.9 and 87.9, 25 ppm: 0 and 4.4, 100 ppm: 0 and 0.

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