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A revision of the ^{13}C NMR spectral assignment of globulol

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Abstract. Complete assignment of the ^{13}C NMR spectrum of the aromadendrane-type sesquiterpene alcohol, globulol was reported, however, it still remained to be ambiguous. We report here the unambiguous and complete assignment of the ^{1}H and ^{13}C NMR spectra of (+)-globulol, which was isolated from the liverworts *Plagiochila ovalifolia*, *Neotrichocolea bissetii* and *Pallavicinia subciliata*.

1. Introduction

The first isolation of (−)-globulol was reported from *Eucalyptus globulus* [1]. Our previous work of the terpenoid constituents of liverworts resulted in the first isolation of (+)-globulol (**1**) from *Plagiochila yokogurensis* [2–4]. Further isolation of **1** from the liverwort *Mylia taylorii* [5] was reported. Although the structure of **1** was drawn erroneously [2,3], it was reported that many species of *Plagiochila* (Jungermanniales) contained **1**. Whereas the ^{13}C NMR spectra of globulol have been reported [6,7], the assignments of the data remained to be ambiguous. We report here the unambiguous and complete assignment of the ^{1}H and ^{13}C NMR spectra of **1**, which was isolated from the liverworts *Plagiochila ovalifolia*, *Neotrichocolea bissetii* and *Pallavicinia subciliata*.

2. Experimental

2.1. Plant materials

Plagiochila ovalifolia Mitt. (dry weight: 418.3 g, specimen No. 96089) was collected in June 1996 at Kawakami, Nara, Japan. *Neotrichocolea bissetii* (Mitt.) Hatt. (557.2 g, 97052) was collected in April 1997 at Kamikatsu-cho, Tokushima, Japan. *Pallavicinia subciliata* (Aust.) Steph. (317.5 g, 96052) was collected in May 1996 in Tsushima, Nagasaki, Japan. Voucher specimens are deposited at Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

2.2. Extraction and isolation

The materials were gently washed with water, impurities removed, ground mechanically and then extracted with Et_2O, respectively. The ether extracts of *Plagiochila ovalifolia* (11.5 g), *Neotrichocolea*

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bissetii (13.31 g) and *Pallavicinia subciliata* (8.18 g) were chromatographed on silica gel using n-hexane-ethyl acetate, followed by Sephadex LH-20 eluted with CH_2Cl_2-MeOH (1 : 1 v/v) to give a mixture including **1**. Further purification of the mixture by preparative HPLC afforded (+)-globulol (**1**) as minor constituent of each species (3.9 mg, $[\alpha]_D + 31.4^\circ$ ($CHCl_3$; *c* 0.14) from *P. ovalifolia*, 1.6 mg, $[\alpha]_D + 20.5^\circ$ ($CHCl_3$; *c* 0.08) from *N. bissetii* and 12.7 mg, $[\alpha]_D + 35.9^\circ$ ($CHCl_3$; *c* 1.27) from *P. subciliata*).

2.3. Nuclear magnetic resonance instrumental conditions

The ^1H and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively, on a Varian UNITY 600 spectrometer using $CDCl_3$ with TMS as the internal standard. Measurements were performed at 25°C using 5 mm o.d. sample tubes. For the ^1H–^{13}C correlation experiment, pulsed field gradient heteronuclear single-quantum correlation (GHSQC) was used [8,9]. The spectra were acquired with 1024 data points and 256 time increments with 8 transients per increment. The relaxation delay was 1.5 s and average ^1J(C–H) was set to 140 Hz.

3. Results and discussion

The first report of the assignment of the ^{13}C NMR spectrum of **1** found in the liverwort was demonstrated by Matsuo et al. [5]. Miyazawa et al. [6] further reported the assignment of commercial (−)-globulol (from Fluka). Recently, the assignment [6] has been revised by Wu et al. [7]. Their assignments were given in Table 1. Our assignment of **1** isolated from the liverworts and of commercial (−)-globulol (from Fluka) was not identical to that of the latest reported assignment, although it was satisfactorily identical to the assignment [6] which has been revised by Wu et al. The latest reported assignment was therefore questionable. In order to clarify the assignment of **1**, further experiment seemed to be necessary.

Table 1
The assignments of the ^{13}C NMR spectral data for globulol

Atom	Ref. [5]	Ref. [6]	Ref. [7]	Present work
1	57.0	57.2	56.8	57.0
2	26.1	26.3	26.5	26.1
3	34.6	34.7	28.6*	34.6
4	36.3	36.4	39.5*	36.3
5	39.6	39.7	44.3*	39.7
6	26.7*	28.6	26.1*	28.3
7	28.3*	26.9	28.4*	26.7
8	20.2	20.2	20.1	20.2
9	44.6	44.7	36.3*	44.6
10	75.2	75.1	75.1	75.3
11	19.4	19.3	19.2	19.4
12	20.2	15.8	15.7	15.8
13	15.8	28.7	28.1	28.7
14	28.7	20.2	34.5*	20.1
15	16.0	16.1	16.1	16.0

*This assignment contrasted with the value identified by present work.

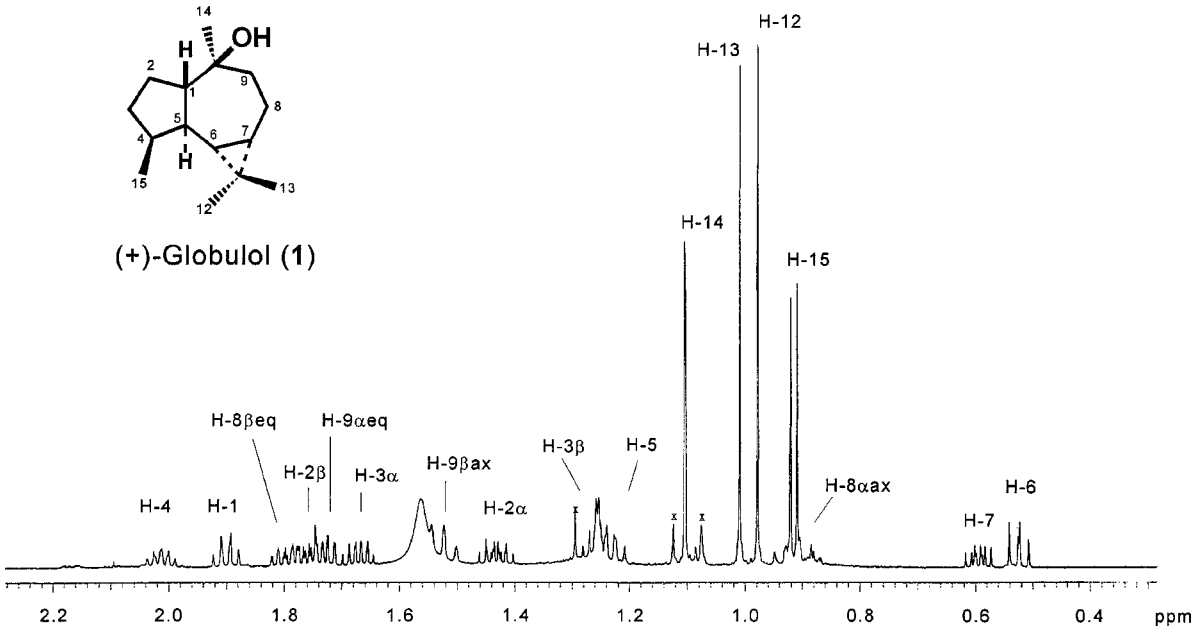


Fig. 1. The ^{1}H NMR spectrum of (+)-globulol (**1**).

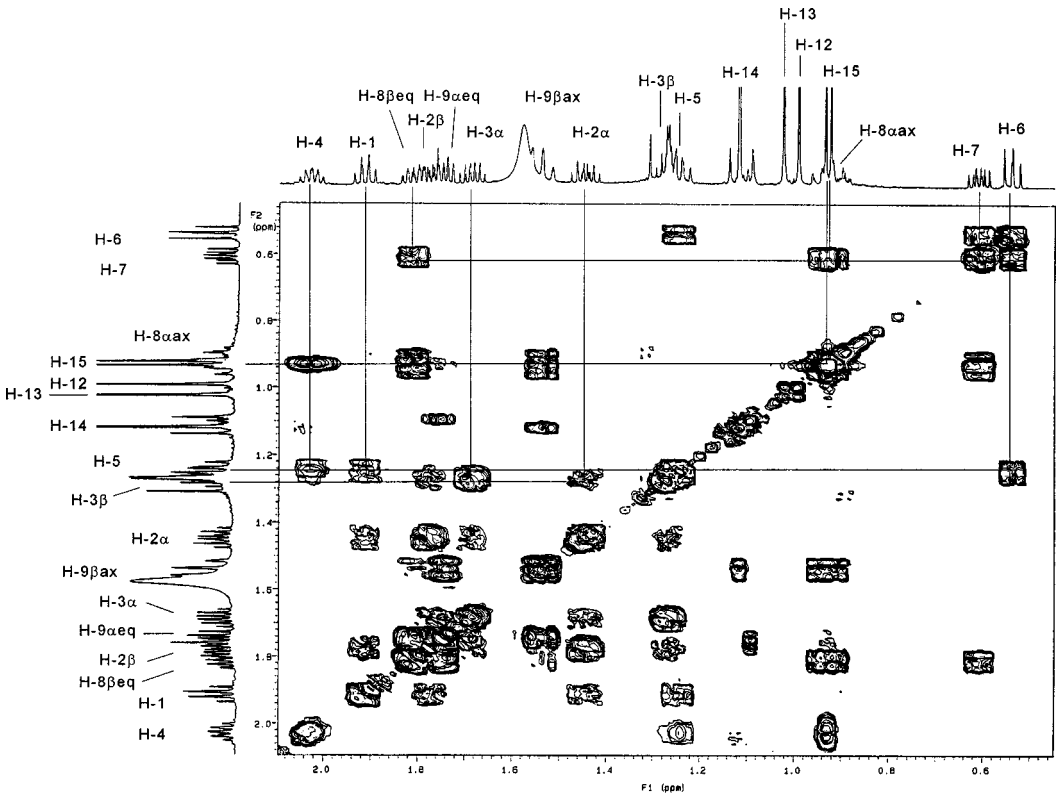


Fig. 2. Homonuclear ^{1}H–^{1}H COSY diagram of (+)-globulol (**1**).

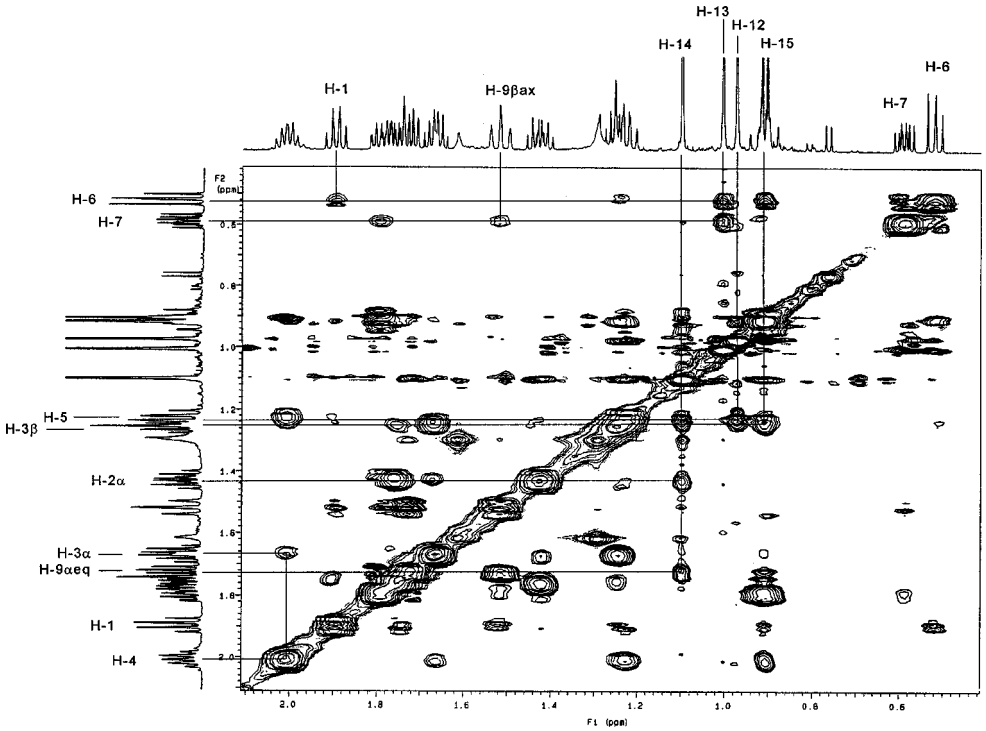


Fig. 3. NOESY spectrum of (+)-globulol (**1**).

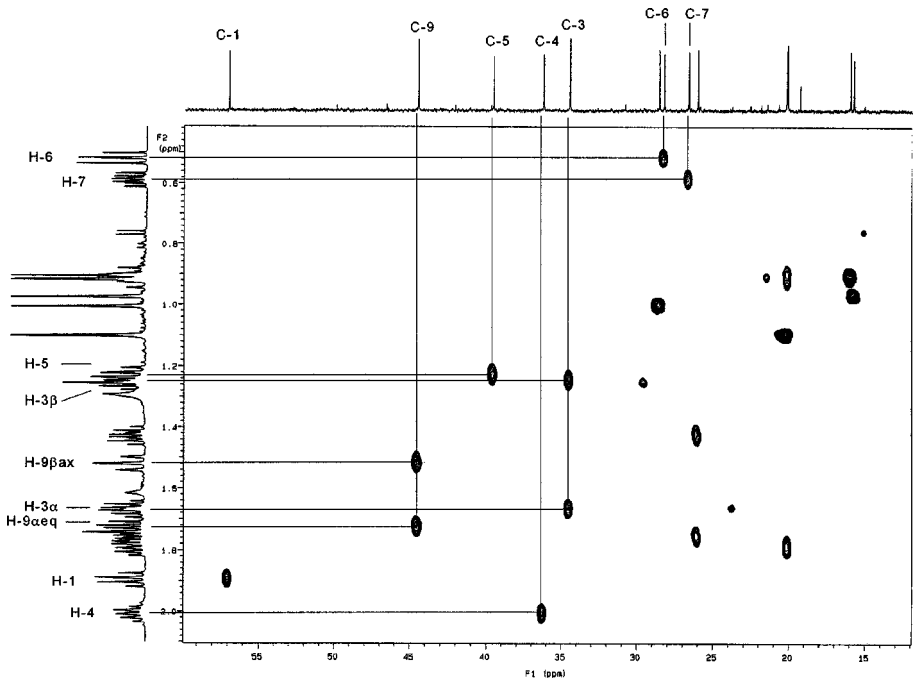


Fig. 4. Pulsed field gradient heteronuclear single-quantum correlation (GHSQC) diagram of (+)-globulol (**1**).

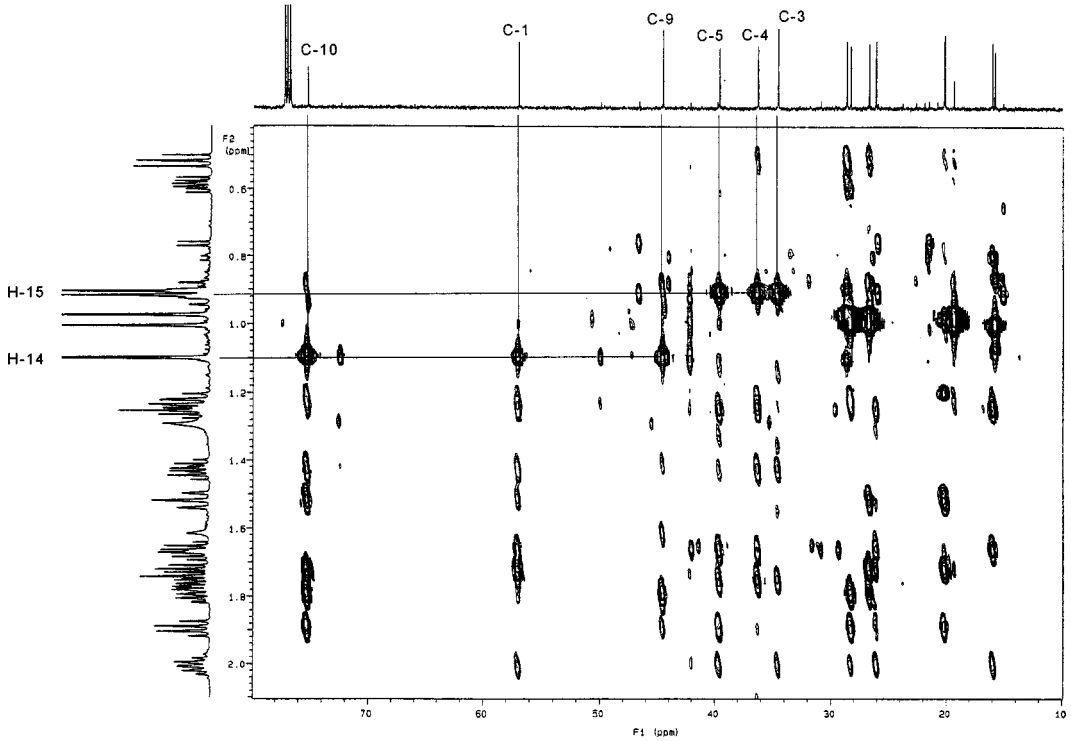


Fig. 5. HMBC spectrum of (+)-globulol (**1**).

The assignment of all protons of **1** (Fig. 1) was carried out by analysis of ^{1}H–^{1}H COSY and NOESY spectra as shown in Figs 2 and 3. Particularly, the connective correlation of H-4, -5, -6 and -7 was carefully assigned, since the carbons connected with those protons were different order (Table 1) in comparison with assignment of the ^{13}C NMR spectral data for **1** reported by Wu et al. Although there are no correlation between H-4 and -3 in Fig. 2, correlations for spin-spin coupling between H-2 and -3 (Fig. 2), and for NOEs between H-4 and -3α, and H-15 and -3β (Fig. 3) were observed in ^{1}H–^{1}H COSY and NOESY spectra. The assignment of all protons was apparent from above spectral evidences. The ^{1}H–^{13}C chemical shift correlation experiment allowed the assignment of all protonated carbons (Fig. 4). Two quaternary carbons C-10 (δ_c 75.3 ppm) and 11 (δ_c 19.4 ppm) were clearly distinguishable from their chemical shifts. Further confirmation of the assignment was provided by the HMBC spectrum of **1** (Fig. 5). The ^{13}C NMR chemical shift values are summarized in Table 1.

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