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Determination of the absolute stereochemistry of lupane triterpenoids by fucofuranoside method and ORD spectrum

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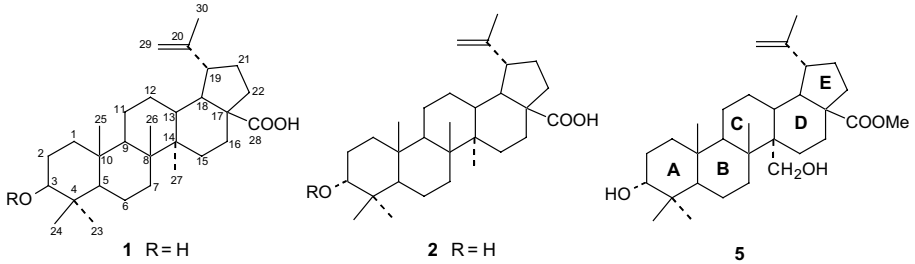
This paper is dedicated to the late Professor Yingjie Chen (deceased November 18, 2003)

Abstract—The absolute configurations of the secondary alcohols at C-3 position in betulinic acid and 3-epibetulinic acid have been unambiguously determined as *S*- and *R*-configurations, respectively, based on the fucofuranoside method. The absolute stereochemistry of 3α, 27-dihydroxylupen-20(29)-en-28-oic acid methyl ester, a potent topoisomerase II inhibitor, was determined to be 3*R*, 5*R*, 8*R*, 9*R*, 10*R*, 13*R*, 14*S*, 17*S*, 18*R*, and 19*R* by NMR and ORD spectroscopic studies.

The lupane-type triterpenes are one of three major pentacyclic triterpenoids in the natural sources, and showed potent anti-cancer and anti-HIV activities.[1–6] The first lupane triterpene, butulin, was obtained from the bark of *Betula alba* in 1788,[7] but the correct structure of lupane triterpene was proposed only in 1951 through correlation of lupane with β-amyrin.[8] Generally the stereochemistry of lupane triterpenes was established according to the biogenetic route from epoxy squalene proposed by Ruzicka and co-workers[9,10] and the relative configuration of lupane triterpenoids was confirmed by chemical transformation[11] and X-ray analysis,[12] but the absolute configuration proposed on the basis of the β-configuration at the C-3 position was not conclusive.[12]

In 1971, Stork et al. reported first total synthesis of (±)-lupeol, a representative lupane triterpene, in a highly stereoselective fashion, while the absolute stereochemistry of natural lupeol remained unsolved.[13] Since there is no clear evidence to unambiguously determine the absolute stereochemistry of lupane-type triterpenes, we decided to investigate the absolute configuration of the secondary alcohols at C-3 position in lupane triterpenoids by the fucofuranoside method designed by Kobayashi.[14] The fucofuranoside method is suitable for determining the absolute configuration of a chiral secondary or a tertiary alcohol through transformation of the alcohols to the β-D- and β-L-fucofuranosides.[14–16] In this paper, we report the determination of the absolute



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configuration of betulinic acid (**1**) and 3-epibetulinic acid (**2**) with the aid of fucofuranoside method and further the absolute configuration of 3α, 27-dihydroxylupen-20(29)-en-28-oic acid methyl ester (**5**) by NMR and ORD spectra.

Betulinic acid (**1**), 3-epibetulinic acid (**2**) and their acetates (**3** and **4**) have been isolated from under ground parts of *Peganum nigellastrum*.[1] The relative stereochemistry at the C-3 position of these compounds is clear from ^{1}H and ^{13}C NMR data. In the ^{1}H NMR spectra, the C-3-H of **1** and **3** resonated at δ 3.00 (dd, $J = 4.8$ and 11.2 Hz) and δ 4.46 (dd, $J = 6.1$ and 10.2 Hz), respectively, showing the J value fitting for axial orientation. On the other hand, those of **2** and **4** resonated δ 3.39 (t, $J = 2.8$ Hz) and δ 4.60 (br s), respectively, with equatorial orientation. Furthermore, Wiemer et al. reported that the ^{13}C resonance of the axial methyl group at the C-4 position of β-amyrin derivatives is strongly influenced by the stereochemistry of the hydroxyl group at C-3.[17] Indeed, the axial methyl carbon (C-24) of **1** showed upper field shift (Δ 6.1 ppm) compared with that of **2** (Table 1). The ^{13}C resonances of C-1 and C-5 of **1** also shifted to lower field as compared with those of **2** on account of γ-effect (Table 1). The similar behaviors in the ^{13}C NMR spectra were also observed in **3** and **4** (Table 1). Thus the relative configuration at C-3 position of **1**–**4** were established.

Table 1. ^{13}C NMR chemical shift (ppm, 100 MHz) of the A ring carbons in **1**–**5**[a]

C no	1	2	3	4	5	1a	1b	2a	2b
1	38.7	33.3	38.3	33.9	33.8	39.2	38.7	34.2	34.8
2	27.4	25.5	23.7	22.9	25.7	26.8	22.9	21.0	25.0
3	78.8	76.3	80.3	78.4	76.5	87.2	81.8	79.2	84.0
4	38.9	37.1	37.1	36.6	37.9	39.5	38.9	37.7	38.4
5	55.4	49.1	55.4	50.2	49.6	56.0	56.2	49.9	49.9
10	37.2	37.5	37.8	37.2	37.9	37.3	37.5	37.6	37.7
23	27.9	28.3	27.9	27.8	28.5	28.3	28.7	29.4	28.8
24	16.0	22.1	16.0	21.7	22.4	17.0	17.1	22.8	22.6
25	15.9	15.9	16.3	15.9	16.8	16.4	16.4	16.4	16.4
1′	—	—	—	—	—	111.6	105.8	106.1	111.4

[a] **1**–**5** were measured in $CDCl_3$; **1a**, **1b**, **2a**, and **2b** were measured in C_5D_5N.

With the aid of fucofuranoside method,[14] the absolute stereochemistry of lupane-type triterpenes, betulinic acid (**1**) and 3-epibetulinic acid (**2**) were determined. Conversion of betulinic acid (**1**) to methyl betulinate with diazomethane followed by glycosidation with 1-bromofucofuranose triacetate and then hydrolysis[15] afforded the β-D-fucofuranoside (**1a**) and β-L-fucofuranoside (**1b**) (Fig. 1). The ^{13}C NMR spectra of two diastereomeric furanosides were recorded in pyridine-d_5, and the chemical shifts were assigned by extensive 2D NMR spectra (Table 1). In the same way, 3-epibetulinic acid yielded diastereomeric β-D-fucofuranoside (**2a**) and β-L-fucofuranoside (**2b**), respectively. The $\Delta\delta_C$ ($\delta_C^L - \delta_C^D$) values were obtained by subtracting the chemical shifts of the β-D-isomer from the corresponding chemical shifts of β-L-isomer, and the results were shown in Figure 2. When the two glycosides are viewed according to Kobayashi's method, placing the furanosyl group in front and the carbinyl proton down, the $\Delta\delta_C$ values of anomeric carbon, α-carbon and left-hand β-carbon in **1** are significantly negative, whereas the change is small for the right-hand β-carbon. The $\Delta\delta_C$ values of anomeric carbon, α-carbon and right-hand β-carbon in **2** are significantly positive, but the change is small for the left-hand β-carbon. From the above data, the absolute configurations at C-3 position in betulinic acid (**1**) and 3-epibetulinic acid (**2**) are unambiguously determined to be *S*-configuration and *R*-configuration, respectively.

3α, 27-Dihydroxylupen-20(29)-en-28-oic acid methyl ester (**5**) isolated from *P. nigellastrum* is a potent topoisomerase II inhibitor.[1] The chemical shift and coupling pattern of C-3-H (δ 3.38, t, $J = 2.8$ Hz) and ^{13}C NMR chemical shifts of A ring carbons (Table 1) are strictly

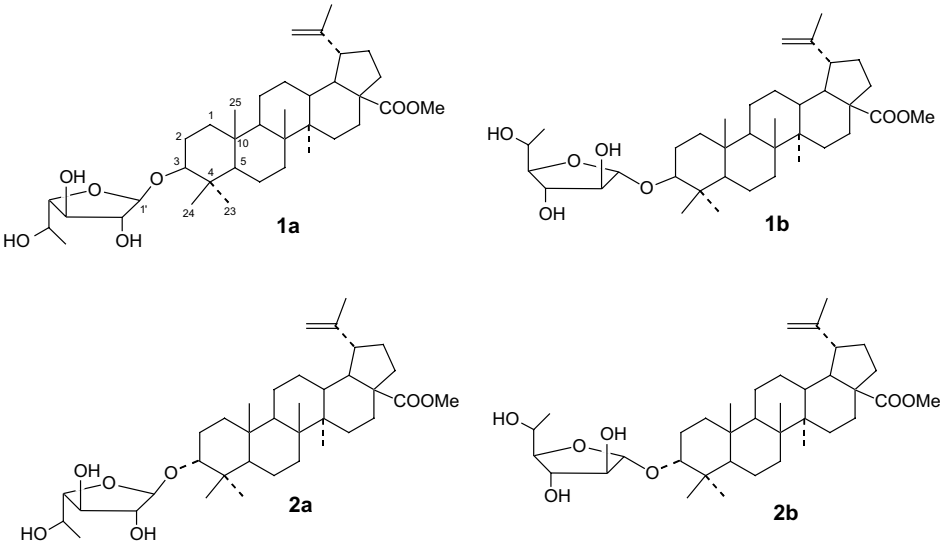

Figure 1. The β-D- and β-L-fucofuranoside derivatives of **1** and **2**.

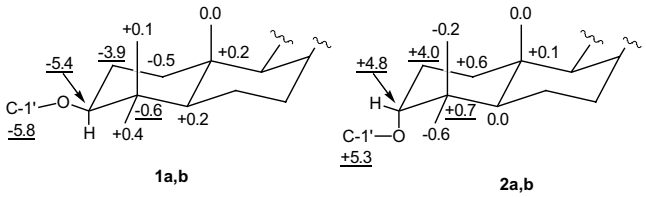

Figure 2. The $\Delta\delta_C$ ($\delta_C^L - \delta_C^D$) values were observed for the carbons of the β-D- and β-L-fucofuranoside derivatives of the secondary alcohols **1** and **2**.

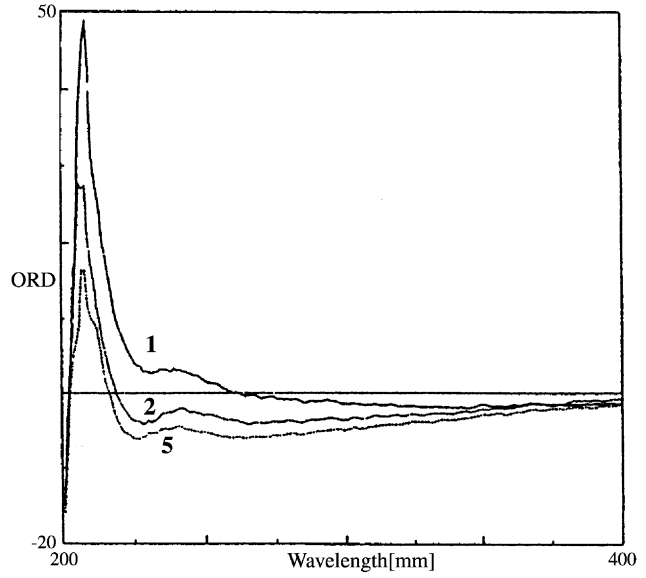

Figure 3. ORD spectrum of **1**, **2**, and **5**.

similar to those of 3-epibetulinic acid (**2**), indicating the hydroxyl group to be axial orientation. The A/B, B/C, C/D, and D/E ring junctions were completely elucidated as all-*trans* based on the NOESY spectrum[1] and chemical conversion.[18] In order to determine the absolute stereochemistry of **5**, the optical rotatory dispersion (ORD) spectrum of **5** was compared with those of **1** and **2** at the same concentration (0.2 mg/mL) in methanol. On the basis of the ORD spectral similarities of **2** and **5** (Fig. 3), the 3-*R* configuration was assigned for **5**. Thus, the absolute stereochemistries of 10 asymmetric centers in **5** are determined to be 3*R*, 5*R*, 8*R*, 9*R*, 10*R*, 13*R*, 14*S*, 17*S*, 18*R*, and 19*R*.

In conclusion, the present study gives a concise and effective method for the determination of the absolute configuration of lupane triterpenoids. The results showed that the absolute stereochemistry of lupane triterpenoids is consistent with the conventional biosynthetic pathway.[9,10]

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