Chem. Pharm. Bull. 28(3)1006—1008(1980)

^{13}C Nuclear Magnetic Resonance of Lupane-Type Triterpenes, Lupeol, Betulin and Betulinic Acid

Mochammad Sholichin, Kazuo Yamasaki, Ryoji Kasai, and Osamu Tanaka

Institute of Pharmaceutical Sciences, Hiroshima University, School of Medicine[1]

(Received October 19, 1979)

The ^{13}C NMR apectra of lupane-type triterpenes, lupeol (**1**), its acetate (**2**), betulin (**3**) and betulinic acid (**4**), were recorded and the signals assigned.

Keywords——^{13}C NMR; lupane; triterpene; lupeol; betulin; substitution induced shift; PRFT

The ^{13}C nuclear magnetic resonance (^{13}C NMR) assignments of a variety of triterpene skeletons have recentyl been elaborated for the purpose of application to biosynthetic studies and structure determination. However, no report of a ^{13}C NMR study on the fundamental lupane-type triterpenes has appeared in the literature as far as we know. Further to our ^{13}C NMR study of dammarane-type triterpenes,[2] the persent paper deals with the assignments of carbon resonances of typical lupane-type triterpenes, lupeol (**1**), its acetate (**2**), betulin (**3**) and betulinic acid (**4**).

The signal assignments were carried out by means of single frequency off-resonance decoupling techiques, partially relaxed Fourier transform (PRFT) studies and comparison with the reported data for dammarane- and germanican-type triterpenes. Comparison of the spectra of **1**, **2**, **3** and **4** in the light of substitution induced shifts was also useful for determination of the shift allocation.[3] The δ-values recorded in $CDCl_3$ for **1**—**3** and in $CDCl_3$–C_5D_5N for **4** are listed in Table I.

The signals due to carbons on the A-, B- and C-rings and C-15 on the D-ring were readily characterized by comparison with the corresponding signals of germanicol (**5**)[4] and dammarenediol-II (**6**)[2] both of which have the same partial structure as the compounds in the present study. The olefinic carbon signals (C-20 and -30) of all the compounds (**1**—**4**) were identified on the basis of the chemical shifts and the multiplicities. The remaining singlet of **1** at δ 43.0 which was displaced downfield by +4.9 ppm in the spectrum of **3** due to the hydroxy-substitution at C-28, could be assigned to the C-17 quaternary carbon, though this assignment may be interchanged with that of C-14 (δ 42.9).

A triplet signal (–CH_2–) of **1** at δ 35.6 was assigned to C-16 from the calculated value derived from the semiempirical equation reported by Beierbeck *et al.*[4] This was confirmed by its upfield shift (−6.4 ppm)[5] in the spectrum of **3** due to the hydroxy substitution at C-28. Other triplets of **1** at δ 29.9 and 40.0 were assigned to C-21 and -22, respectively, since on going from **1** to **3**, the latter signal was shielded by −6.0 ppm, while the former remained almost unaffected.

With regard to seven methyl carbon resonances (quartets) of **1**, the signals due to C-23, -24, -25, -26 and -27 were identified by analogy with the assignments of the corresponding resonances for **5** and **6**. The characterization of the C-24 signal was also substantiated by

1) Location: *1-2-3, Kasumi, Hiroshima 734, Japan.* Correspondence should be addressed to O. Tanaka.
2) J. Asakawa, R. Kasai, K. Yamasaki and O. Tanaka, *Tetrahedron*, **33**, 1935 (1977).
3) J.B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, 1972.
4) H. Beierbeck, J.K. Saunders and J.W. ApSimon, *Can. J. Chem.* **55**, 2813 (1977).
5) A negative sign denotes an upfield shift on substitution.

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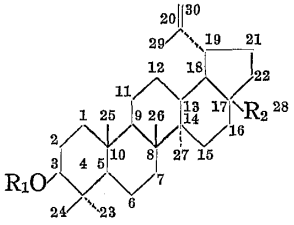
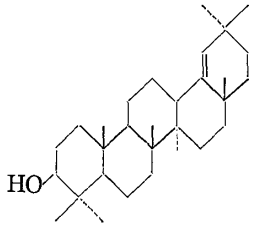
the deshilding (+1.2 ppm) on acetylation of the 3β-hydroxy group (acetylation shift; **1** to **2**).[6] The identification of the remaining two methyl signals, C-28 and -29 was achieved as follows. The signal of **1** at δ 18.0 was strongly deshielded to δ 60.6 on conversion to **3**, and was assigned to C-28, while the other signal of **1** at δ 19.3 remained essentially unshifted, being identified as C-29. The assignment of C-29 was confirmed by aPRFT experiment with **2**; the C-29 methyl carbon on the side chain must have faster segment molecular movement than any other methyl carbon on the ring skeleton, being readily differentiated from others because of its larger T_1 value in the PRFT inspection.[7]

The signal assignments of **4** were readily achieved on the basis of the above results and the substitution-induced shift of the carboxyl group at C-28.



1: R_1=H, R_2=CH_3
2: R_1=Ac, R_2=CH_3
3: R_1=H, R_2=CH_2OH
4: R_1=H, R_2=COOH



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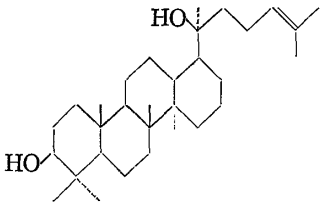

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Chart 1

TABLE I. The ^{13}C NMR Chemical Shifts of Lupane-Type Triterpenes[a]

	1	2	3	4		1	2	3	4
C-1	38.7	38.4	38.7	39.0	C-17	43.0[b]	43.0[b]	47.9	56.3
C-2	27.5	23.7	27.4	27.6	C-18	48.0[c]	48.0[c]	47.9	47.1
C-3	79.0	81.0	79.1	78.2	C-19	48.3[c]	48.3[c]	48.8	49.4
C-4	38.9	37.8	38.8	39.0	C-20	150.9	150.9	150.5	150.9
C-5	55.3	55.4	55.3	55.5	C-21	29.9	29.9	29.8	29.9
C-6	18.3	18.2	18.3	18.4	C-22	40.0	40.0	34.0	37.3
C-7	34.3	34.3	34.3	34.5	C-23	28.0	28.0	28.1	28.2
C-8	40.9	40.9	41.0	40.8	C-24	15.3	16.5	15.4	15.6
C-9	50.5	50.4	50.5	50.7	C-25	16.1[d]	16.2[d]	16.1	16.1
C-10	37.2	37.1	37.3	37.3	C-26	16.0[d]	16.0[d]	16.1	16.1
C-11	21.0	21.0	20.9	21.0	C-27	14.6	14.5	14.8	14.7
C-12	25.2	25.1	25.2	25.6	C-28	18.0	18.0	60.6	178.9
C-13	38.1	38.1	37.2	38.2	C-29	19.3	19.3	19.1	19.4
C-14	42.9[b]	42.9[b]	42.8	42.5	C-30	109.3	109.4	109.7	109.4
C-15	27.5	27.5	27.1	30.8	Ac		21.3		
C-16	35.6	35.6	29.2	32.6			170.8		

a) Solvent $CDCl_3$: **1**—**3**, $CDCl_3$-C_5D_5N(1:1): **4**.
b, c, d) Assignments bearing the same superscript in any one spectrum may be reversed.

Experimental

Materials——Compounds **1** and **2** were isolated from the leaves of *Stevia rebaudiana*.[8] Compound **3** was isolated from the bark of *Betula mandshurica*. The sample of **4** was provided by Prof. I. Nishioka of Kyushu University, to whom we are very grateful.

NMR Spectral Measurements——A JEOL PFT-100 spectrometer equipped with an EC-6 computer was used. All chemical shifts were calculated from internal TMS. ^{13}C FT-NMR spectra were recorded at

6) S. Seo, Y. Tomita and K. Tori, *Tetrahedron Lett.*, **1975**, 7.
7) A. Allerhand, D. Doddrel and R. Komoroski, *J. Chem. Phys.*, **55**, 189 (1971).
8) M. Sholichin, K. Yamasaki, R. Miyama, S. Yahara and O. Tanaka, *Phytochemistry*, **19**, 326 (1980).

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25.15 MHz at 26° and at a concentration of 0.1—0.21 M in 10 mm tubes. Condition of FT-NMR measurements were; spectral width, 5 KHz; pulse interval, 1 sec; pulse flipping angle, 45°; acquisition time, 0.4 sec; number of data points, 4096; computer-limited resolution, 0.1 ppm, number of transients, 500—1000. The PRFT spectrum of **2** was observed with the pulse sequence of $\{180° - 0.5 \text{ sec} - 90° - 5.5 \text{ sec}\}_{4800}$ at a concentration of 0.21 M.

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