Journal of Natural Products
Vol. 52, No. 3, pp. 595-605, May-Jun 1989

^{13}C-NMR CORRELATION OF STEREOCHEMISTRY IN LANOSTANOID TRITERPENES

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ABSTRACT.—A series of lanostane-type triterpenes isolated from *Ganoderma lucidum* were identified as pairs of positional or stereo-isomers. Comparison of the ^{13}C chemical shifts among these structurally related compounds allowed several empirical rules to be formulated. The correlation between ^{13}C chemical shifts and stereochemical features was evident based on this empirical analysis.

The fungus *Ganoderma lucidum* (Fr.) Karst (Polyporaceae), used in traditional Chinese medicine, has attracted great attention recently because of production of many highly oxygenated triterpenes and sterols with various biological activities (1–5). Biogenetically, the majority of the approximately 100 oxygenated triterpenes are derived from the lanostanoid skeleton with oxygenated functionalities mainly at the C-3, C-7, C-15, C-22, C-23, and C-26 carbons. Most interestingly from the structural and biosynthetic point of view, many triterpenes have been identified as pairs of stereo- or positional isomers, particularly at C-3 (Figure 1). Accumulating ^{13}C- and ^{1}H-nmr spectroscopic information about these structurally related compounds allowed us to formulate several empirical rules for ^{13}C-nmr signal assignments. Both for the characterization of new, related compounds and future biosynthetic studies, we report here the empirical rules which correlate stereochemical features and ^{13}C chemical shifts.

RESULTS AND DISCUSSION

We and other investigators have established that the A/B rings of triterpenes reported in Figure 1 are in a trans, chair-like configuration (4, 6–11). In this lanosta-7,9(11),24-trien-26-oic acid series, the stereochemistry of several compounds was also confirmed by single crystal X-ray studies (4,12). The configuration of substituents at C-3 affected the ^{13}C chemical shifts of adjacent carbons in a consistent and predictable way. Based on the comparison of more than eight pairs of C-3 epimers and structural analogues, we confirmed that C-3 carbons bearing equatorial substituents, -OH and -OAc, were more deshielded than those with axial substituents (7–11). The difference of C-3 chemical shifts due to this configurational difference was in the range of 2–3 ppm (Table 1). This observation was based on the comparison of C-3 signals of the 3α series of compounds **1**, **3**, **4**, **8**, **12**, **18**, **21**, **23** to those of the 3β series of compounds **2**, **5**, **6**, **9**, **13**, **19**, **20**, **22**, respectively. The configuration at C-3 has an even more profound steric effect on the C-29 and C-30 carbon signals (Table 1). A general observation was that 3β substituents, hydroxy as well as acetoxy, brought about a large upfield shift of the C-30 carbon signal by about 5–7 ppm relative to the 3α-substituted counterparts (Table 1). The C-29 signals, at 27–28 ppm, of all C-3 epimers remained for the most part undisturbed. This trend was valid for all relevant compounds in Figure 1. Epimerization of C-3 substituents also affected other carbons in the vicinity. The C-1 carbon of 3α-substituted triterpenes resonated at about 30 ppm and was shifted 5 ppm downfield in the 3β-substituted counterparts (Table 1). The same trend was also observed at C-5. Thus the C-5 signals were at ∼43 ppm in the 3α-substituted series and were at ∼48 ppm in the 3β-substituted series. Acetylation of the corresponding C-3 hydroxy groups

TABLE 1. ^{13}C-nmr Spectral Data of Compounds **1–24**.

Carbon	Compound					
	1	**2**	**3**	**4**[a]	**5**[a]	**6**[a]
C-1	30.51 t	35.26 t	29.78 t	30.53 t	35.61 t	35.28 t
C-2	23.03 t	24.06 t	25.44 t	23.04 t	27.62 t	24.09 t
C-3	77.98 d	80.60 d	75.97 d	78.00 d	78.80 d	80.66 d
C-4	36.42 s	37.43 s	37.20 s	36.41 s	38.54 s	37.43 s
C-5	43.82 d	48.83 d	42.81 d	43.93 d	48.74 d	48.94 d
C-6	22.70 t	22.69 t	22.85 t	22.69 t	22.88 t	22.63 t
C-7	121.04 d	120.93 d	121.17 d	121.10 d	121.28 d	121.02 d
C-8	140.09 s	140.01 s	140.08 s	140.66 s	140.04 s	140.64 s
C-9	145.40 s	145.52 s	145.86 s	145.98 s	145.83 s	145.73 s
C-10	37.22 s	37.16 s	37.23 s	37.21 s	37.33 s	37.15 s
C-11	115.52 d	116.00 d	115.45 d	115.52 d	115.76 d	116.01 d
C-12	37.87 t	37.86 t	37.23 t	37.21 t	37.33 t	37.15 t
C-13	44.04 s	43.92 s	43.99 s	44.33 s	43.99 s	44.21 s
C-14	51.30 s	51.19 s	51.29 s	51.95 s	51.19 s	51.83 s
C-15	77.26 d	77.15 d	77.29 d	74.57 d	77.25 d	74.48 d
C-16	36.89 t	36.84 t	36.91 t	39.89 t	36.89 t	39.77 t
C-17	48.76 d	48.67 d	48.74 d	48.71 d	48.74 d	48.67 d
C-18	15.85 q	15.82 q	15.85 q	15.84 q	15.85 q[c]	15.82 q[c]
C-19	22.52 q[c]	22.69 q	22.66 q[c]	22.56 q[c]	22.71 q	22.74 q
C-20	35.85 d	35.79 d	35.85 d	35.81 d	35.84 d	35.76 d
C-21	18.08 q	18.03 q	18.06 q[d]	18.17 q[d]	18.09 q[d]	18.12 q[d]
C-22	34.54 t	34.49 t	34.55 t	34.62 t	34.54 t	34.67 t
C-23	25.82 t	25.78 t	25.83 t	25.75 t	25.83 t	25.70 t
C-24	144.99 d	144.90 d	145.00 d	145.13 d	144.94 d	145.01 d
C-25	126.72 s	126.72 s	126.64 s	126.77 s	126.74 s	126.81 s
C-26	172.92 s	173.01 s	172.52 s	172.86 s	172.86 s	172.93 s
C-27	11.84 q	11.78 q	11.88 q	11.89 q	11.89 q	11.86 q
C-28	18.31 q	18.19 q	18.39 q[d]	17.13 q[d]	18.27 q[d]	16.99 q[d]
C-29	27.65 q	27.97 q	28.07 q	27.66 q	28.06 q	27.95 q
C-30	22.32 q[c]	16.79 q	22.55 q[c]	22.35 q[c]	15.70 q[c]	16.81 q[c]
AcCO	170.65 s	170.82 s	171.12 s	170.75 s	171.07 s	170.90 s
AcCO	171.02 s	171.04 s	—	—	—	—
AcCO	—	—	—	—	—	—
Ac*Me*	21.15 q	21.06 q	21.31 q	21.19 q	21.30 q	21.16 q
Ac*Me*	21.26 q	21.18 q	—	—	—	—
Ac*Me*	—	—	—	—	—	—

[a]Spectra were obtained at 50.3 MHz (Bruker MSL-200).
[b]Samples were dissolved in $CDCl_3$-CD_3OD (ca. 5:1).
[c,d]Tentative assignments and values with same superscript in same column may be interchanged.

TABLE 1. (Continued)

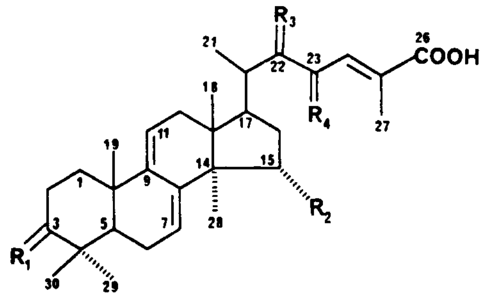
Carbon	Compound					
	7	**8**	**9**[b]	**10**	**11**	**12**
C-1	36.60 t	29.96 t	35.49 t	30.53 t	35.63 t	30.47 t
C-2	37.48 t	25.58 t	27.04 t	23.06 t	27.67 t	22.09 t
C-3	216.59 s	76.27 d	78.30 d	77.99 d	78.80 d	77.93 d
C-4	47.42 s	37.34 s	38.28 s	36.44 s	38.58 s	36.39 s
C-5	50.39 d	43.08 d	48.45 d	44.16 d	48.75 d	43.76 d
C-6	23.63 t	22.96 t	22.61 t	22.70 t	22.91 t	22.69 t
C-7	121.04 d	121.43 d	121.02 d	121.47 d	121.56 d	121.22 d
C-8	140.37 s	140.86 s	140.52 s	140.40 s	139.89 s	139.87 s
C-9	145.00 s	146.32 s	145.90 s	146.10 s	146.02 s	145.85 s
C-10	37.25 s	37.34 s	37.07 s	37.22 s	37.37 s	37.19 s
C-11	116.92 d	115.68 d	115.52 d	115.17 d	115.52 d	115.27 d
C-12	37.99 t	38.51 t	38.15 t	38.35 t	37.93 t	37.84 t
C-13	44.06 s	44.43 s	43.99 s	43.92 s	43.85 s	43.82 s
C-14	51.31 s	52.15 s	51.59 s	51.97 s	51.25 s	51.28 s
C-15	77.23 d	75.75 d	73.90 d	74.40 d	76.89 d	77.12 d
C-16	36.97 t	39.96 t	39.16 t	39.61 t	36.59 t	36.42 t
C-17	48.85 d	48.80 d	48.72 d	45.35 d	45.37 d	45.33 d
C-18	16.00 q	15.93 q	15.53 q[c]	15.71 q	15.73 q[c]	15.62 q
C-19	22.44 q[c]	22.71 q[c]	22.41 q	22.57 q[c]	22.72 q	22.51 q[c]
C-20	35.92 d	35.92 d	35.60 d	39.20 d	39.53 d	39.48 d
C-21	18.16 q[d]	18.26 q[d]	17.86 q[d]	12.70 q	12.57 q	12.60 q
C-22	34.62 t	34.81 t	34.47 t	74.51 d	74.36 d	74.54 d
C-23	25.92 t	25.74 t	25.38 t	31.66 t	31.82 t	31.80 t
C-24	144.53 d	145.22 d	143.26 d	139.15 d	138.94 d	137.62 d
C-25	126.76 s	126.98 s	127.00 s	129.20 s	129.17 s	130.27 s
C-26	172.10 s	172.83 s	170.45 s	172.08 s	171.53 s	172.90 s
C-27	12.04 q	11.98 q	11.71 q	12.19 q	12.19 q	12.31 q
C-28	18.24 q[d]	17.38 q[d]	16.76 q[d]	17.17 q	18.32 q	18.31 q
C-29	25.40 q	28.19 q	27.67 q	27.63 q	28.05 q	27.62 q
C-30	22.14 q[c]	22.81 q[c]	15.40 q[c]	22.39 q[c]	15.68 q[c]	22.30 q[c]
AcCO	171.21 s	—	—	170.54 s	170.49 s	170.67 s
AcCO	—	—	—	170.76 s	170.96 s	170.67 s
AcCO	—	—	—	—	—	170.94 s
Ac*Me*	21.40 q	—	—	20.94 q	20.89 q	20.89 q
Ac*Me*	—	—	—	21.20 q	21.29 q	21.14 q
Ac*Me*	—	—	—	—	—	21.25 q

TABLE 1. (Continued)

Carbon	Compound					
	13	**14**	**15**	**16**	**17**	**18**
C-1	35.36 t	29.88 t	30.51 t	30.62 t	30.54 t	30.58 t
C-2	24.17 t	25.54 t	23.02 t	23.14 t	23.05 t	23.11 t
C-3	80.69 d	76.68 d	78.01 d	78.06 d	78.00 d	78.03 d
C-4	37.55 s	37.33 s	36.43 s	36.52 s	36.43 s	36.50 s
C-5	48.91 d	42.91 d	43.84 d	44.03 d	43.94 d	43.89 d
C-6	22.83 t	22.95 t	22.73 t	22.79 t	22.70 t	22.80 t
C-7	121.32 d	121.49 d	121.26 d	121.37 d	121.23 d	121.21 d
C-8	140.00 s	140.02 s	139.95 s	140.61 s	140.56 s	140.09 s
C-9	145.75 s	145.98 s	145.84 s	146.11 s	146.05 s	145.89 s
C-10	37.28 s	37.33 s	37.25 s	37.33 s	37.22 s	37.30 s
C-11	115.85 d	115.46 d	115.41 d	115.54 d	115.42 d	115.52 s
C-12	37.95 t	37.85 t	37.76 t	38.33 t	38.33 t	37.94 t
C-13	43.87 s	44.14 s	44.09 s	44.51 s	44.26 s	44.10 s
C-14	51.31 s	51.68 s	51.42 s	52.18 s	51.98 s	51.41 s
C-15	77.00 d	77.00 d	77.22 d	74.57 d	74.51 d	77.32 d
C-16	36.63 t	37.17 t	37.05 t	40.05 t	40.15 t	37.21 t
C-17	45.39 d	48.74 d	48.64 d	48.84 d	49.23 d	49.39 d
C-18	15.73 q	15.99 q	15.89 q	15.97 q	15.74 q	15.85 q
C-19	22.83 q	22.64 q[c]	22.53 q[c]	22.66 q[c]	22.54 q[c]	22.64 q[c]
C-20	39.55 d	32.80 d	32.75 d	32.95 d	33.42 d	33.62 d
C-21	12.63 q	19.37 q	19.30 q	19.57 q	19.41 q	19.33 q
C-22	74.37 d	51.51 t	51.54 t	51.88 t	67.02 d	67.17 d
C-23	31.88 t	201.57 s	201.44 s	201.75 s	43.57 t	43.33 t
C-24	139.03 d	133.83 d	133.92 d	134.09 d	144.80 d	144.66 d
C-25	129.17 s	139.48 s	139.36 s	139.34 s	128.31 s	128.07 s
C-26	171.28 s	171.21 s	171.82 s	170.99 s	171.95 s	171.18 s
C-27	12.31 q	14.09 q	13.92 q	14.09 q	12.64 q	12.78 q
C-28	18.38 q	18.51 q	18.33 q	17.23 q	17.12 q	18.43 q
C-29	28.07 q	28.18 q	27.66 q	27.77 q	27.65 q	27.77 q
C-30	16.91 q	22.64 q[c]	22.33 q[c]	22.46 q[c]	22.34 q[c]	22.44 q[c]
AcCO	170.01 s	171.04 s	170.72 s	170.86 s	170.72 s	170.81 s
AcCO	170.63 s	—	171.08 s	—	—	170.64 s
AcCO	171.12 s	—	—	—	—	—
Ac*Me*	21.01 q	21.39 q	21.16 q	21.30 q	21.16 q	21.41 q
Ac*Me*	21.30 q	—	21.23 q	—	—	21.29 q
Ac*Me*	21.41 q	—	—	—	—	—

TABLE 1. (Continued)

Carbon	Compound					
	19	**20**[b]	**21**	**22**[b]	**23**[b]	**24**
C-1	35.38 t	35.62 t	29.92 t	35.63 t	29.77 t	29.90 t
C-2	24.20 t	27.31 t	25.55 t	27.36 t	25.35 t	25.58 t
C-3	80.71 d	78.58 d	76.07 d	78.61 d	75.82 d	76.19 d
C-4	37.57 s	38.50 s	37.35 s	38.51 s	37.17 s	37.37 s
C-5	48.95 d	48.91 d	43.03 d	48.95 d	42.89 d	43.20 d
C-6	22.86 t	22.81 t	22.94 t	22.83 t	22.79 t	22.98 t
C-7	121.15 d	121.62 d	121.71 d	121.21 d	121.22 d	120.18 d
C-8	140.15 s	140.40 s	140.51 s	140.68 s	140.62 s	142.59 s
C-9	145.67 s	146.17 s	146.31 s	146.02 s	146.10 s	145.94 s
C-10	37.30 s	37.27 s	37.35 s	37.28 s	37.17 s	37.23 s
C-11	116.06 d	115.39 d	115.25 d	115.82 d	115.40 d	115.88 d
C-12	37.99 t	38.32 t	38.47 t	38.40 t	38.30 t	37.78 t
C-13	44.06 s	44.00 s	44.22 s	44.09 s	44.16 s	43.81 s
C-14	51.35 s	51.83 s	52.08 s	51.90 s	51.92 s	50.42 s
C-15	77.08 d	73.99 d	74.58 d	74.25 d	74.23 d	27.90 t
C-16	37.21 t	39.12 t	39.75 t	38.65 t	39.77 t	31.47 t
C-17	49.39 d	45.29 d	45.41 d	45.00 d	49.18 d	50.84 d
C-18	15.87 q	15.59 q	15.78 q	15.70 q	15.67 q	15.66 q
C-19	22.86 q	22.67 q	22.68 q[c]	22.70 q	22.55 q[c]	22.59 q[c]
C-20	33.62 d	39.12 d	39.28 d	40.73 d	33.26 d	36.15 d
C-21	19.34 q	12.54 q	12.78 q	12.41 q	19.27 q	18.29 q
C-22	67.20 d	74.87 d	74.58 d	72.06 d	66.47 d	34.76 t
C-23	43.36 t	31.53 t	31.74 t	34.76 t	43.39 t	25.90 t
C-24	144.67 d	137.23 d	139.20 d	139.91 d	143.62 d	145.66 d
C-25	128.06 s	129.85 s	129.10 s	128.95 s	128.55 s	126.53 s
C-26	171.22 s	171.10 s	170.99 s	170.42 s	170.29 s	172.40 s
C-27	12.81 q	12.31 q	12.34 q	11.42 q	12.69 q	11.99 q
C-28	18.35 q	17.07 q	17.33 q	17.11 q	17.04 q	25.68 q
C-29	28.11 q	27.91 q	28.14 q	27.95 q	28.00 q	28.20 q
C-30	16.94 q	15.59 q	22.77 q[c]	15.70 q	22.66 q[c]	22.79 q[c]
AcCO	170.47 s	169.90 s	170.62 s	—	—	—
AcCO	171.00 s	—	—	—	—	—
AcCO	—	—	—	—	—	—
Ac*Me*	21.32 q	20.87 q	21.03 q	—	—	—
Ac*Me*	21.43 q	—	—	—	—	—
Ac*Me*	—	—	—	—	—	—



	R_1	R_2	R_3	R_4
1	···OAc ▸H	OAc	H_2	H_2
2	···H ▸OAc	OAc	H_2	H_2
3	···OH ▸H	OAc	H_2	H_2
4	···OAc ▸H	OH	H_2	H_2
5	···H ▸OH	OAc	H_2	H_2
6	···H ▸OAc	OH	H_2	H_2
7	=O	OAc	H_2	H_2
8	···OH ▸H	OH	H_2	H_2
9	···H ▸OH	OH	H_2	H_2
10	···OAc ▸H	OH	···H ▸OAc	H_2
11	···H ▸OH	OAc	···H ▸OAc	H_2
12	···OAc ▸H	OAc	···H ▸OAc	H_2
13	···H ▸OAc	OAc	···H ▸OAc	H_2
14	···OH ▸H	OAc	H_2	O
15	···OAc ▸H	OAc	H_2	O
16	···OAc ▸H	OH	H_2	O
17	···OAc ▸H	OH	···OH ▸H	H_2
18	···OAc ▸H	OAc	···OH ▸H	H_2
19	···H ▸OAc	OAc	···OH ▸H	H_2
20	···H ▸OH	OH	···H ▸OAc	H_2
21	···OH ▸H	OH	···H ▸OAc	H_2
22	···H ▸OH	OH	···H ▸OH	H_2
23	···OH ▸H	OH	···OH ▸H	H_2
24	···OH ▸H	H	H_2	H_2
25	···OAc ▸H	H	H_2	H_2

FIGURE 1. Structures of lanostanoid triterpenes used for the ^{13}C-nmr correlational study.

per se played no significant role in affecting C-1/C-5 carbons ($\Delta\delta < 1$ ppm) (Table 2). For stereochemical purposes, the γ-effect of C-3 substituent groups on C-1/C-5 signals, which were shifted upfield by ~5 ppm in 3α-substituted series in comparison with the corresponding 3β series, provided the most useful evidence for the assignment of the configuration at C-3. We observed that acetylation of the C-3 hydroxy group affected C-3 signals by ~2 ppm in a downfield direction (Table 2). The corresponding C-2/C-4 carbons were shielded to different extents. Upon acetylation of the 3-OH, the C-2 and C-4 signals were moved upfield by 2.5–5 and ~1 ppm, respectively (Table 2). We observed that acetylation of the 3-OH did not affect C-29/C-30 to any great extent; the difference of chemical shifts was less than ± 1.3 ppm (Table 2).

Substitution of H-15α by a hydroxyl group caused a downfield shift of 43–48 ppm for C-15, as expected. However, C-14 and C-16 were deshielded to quite different ex-

TABLE 2. Differences in ^{13}C Chemical Shifts of Adjacent Carbons Between Compounds with and without Acetylation of the C-3 Hydroxy Group.[a]

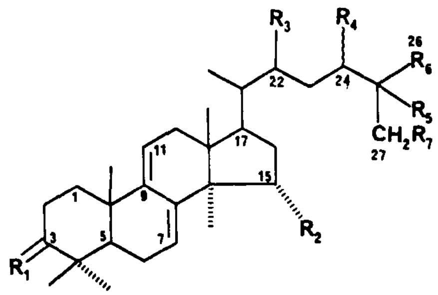
Carbon	Compounds												
	1–3	**1–8**	**1–24**	**2–5**	**2–9**	**12–21**	**12–23**	**13–11**	**13–20**	**15–14**	**2–26**	**2–27**	**28–29**
C-1	0.73	0.55	0.61	−0.35	−0.23	0.55	0.70	−0.27	−0.26	0.63	−1.34	−1.14	0.70
C-2	−2.41	−2.55	−2.55	−3.56	−2.98	−3.46	−3.26	−3.50	−3.14	−2.52	−4.64	−4.84	−2.50
C-3	2.01	1.71	1.79	1.80	2.30	1.86	2.11	1.89	2.11	1.33	2.50	2.50	2.00
C-4	−0.78	−0.92	−0.95	−1.11	−0.85	−0.96	−0.78	−1.03	−0.95	−0.90	−1.97	−1.97	−0.70
C-5	1.01	0.74	0.62	0.09	0.38	0.73	0.87	0.16	0.00	0.93	−0.97	−0.97	0.90
C-29	−0.42	−0.54	−0.55	−0.09	0.30	−0.52	−0.38	0.02	0.16	−0.52	−0.83	−0.93	−0.40
C-30	−0.23	−0.49	−0.47	1.09	1.39	−0.47	−0.36	1.23	1.32	−0.31	0.79	0.79	0.00

[a]Table entries are shift differences ($\Delta\delta$).

TABLE 3. Differences in ^{13}C Chemical Shifts of Adjacent Carbons Between Compounds with and without Acetylation of the C-15 Hydroxy Group.[a]

Carbon	Compounds										
	1–4	**1–8**	**2–6**	**2–9**	**12–10**	**15–16**	**12–17**	**13–20**	**12–21**	**12–23**	**30–31**
C-13	−0.29	−0.39	−0.29	−0.07	−0.10	−0.42	−0.44	−0.13	−0.40	−0.34	−0.30
C-14	−0.65	−0.85	−0.64	−0.40	−0.69	−0.76	−0.70	−0.52	−0.80	−0.64	−0.60
C-15	2.69	1.51	2.67	3.25	2.72	2.65	2.61	3.01	2.54	2.89	2.80
C-16	−3.00	−3.07	−2.93	−2.32	−3.19	−3.00	−3.73	−2.49	−3.33	−3.25	−3.10
C-17	0.05	−0.04	0.00	−0.05	−0.02	−0.20	−3.90	0.10	−0.08	−3.85	0.00
C-28	1.18	0.93	1.20	1.43	1.14	1.10	1.19	1.31	0.98	1.27	1.00

[a]Table entries are shift differences ($\Delta\delta$).



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
26	H / OH	H	H	OH	OH	CH_2OH	H
27	H / OH	H	H	$\Delta^{24(25)}$		CH_2OH	OH
28	OAc / H	H	OAc	$\Delta^{24(25)}$		COOH	H
29	OH / H	H	OAc	$\Delta^{24(25)}$		COOH	H
30	OAc / H	OAc	H	$\Delta^{24(25)}$		COOH	H
31	OAc / H	OH	H	$\Delta^{24(25)}$		COOH	H

FIGURE 2. Structures of compounds **26–31**. The ^{13}C-nmr data of **26** (ganoderiol A) were taken from Sato *et al.* (13); ^{13}C-nmr data of **27** (ganodermatriol) were from Arisawa *et al.* (15); ^{13}C-nmr data of **28** (ganoderic acid R) and **29** (ganoderic acid S) were from Hirotani *et al.* (4); and ^{13}C-nmr data of **30** (ganoderic acid Me) and **31** (ganoderic acid Mf) were from Nishitoba *et al.* (14).

tents, 1.7 ppm for C-14 and ~8.5 ppm for C-16. It is quite interesting that upon hydroxylation at C-15 to give the α epimer, the C-28 carbon turned out to be more shielded and its ^{13}C signal moved upfield by ~8 ppm. This trend was found by comparison of **24**, **26**, **27**, **28**, and **29** with all the C-15 substituted compounds in Figure 1. The corresponding C-17 carbon was also shielded but to a lesser extent (~2 ppm) (Table 1). Acetylation of the 15α hydroxyl group consistently caused a 2.5–3.2 ppm downfield shift of C-15. Unequal upfield shifts of the C-14 and C-16 signals, with C-14 changing by less than 1 ppm and C-16 by ~3 ppm, were also observed (7,9). The larger magnitudes in changes of chemical shifts for C-16 relative to C-14 were observed in both C-15α-hydroxylated and C-15α-acetylated triterpenes listed in Table 3. The hydroxylation at C-15α resulted in a significant shielding effect on C-28. However, acetylation of the 15α-hydroxy group deshielded C-28 and caused a 1–1.2 ppm downfield shift. This was probably due to the crowding of the bulky 15α-OAc group on C-28. The reason for the different direction of γ effect due to C-15 substituents, OH or OAc, on C-28 is not clear, although it probably indicates that both overlapping of Van der Waals interaction and substituent crowding are counteracting each other. No 15β-functionalized triterpenes were available for comparison.

β-Hydroxylation at C-22 caused a large downfield shift of C-22 (at ~67.1 ppm) and C-23 (at ~43.5 ppm). The C-22 signal in the 22α-hydroxylated series is 5 ppm more upfield than the corresponding 22β-hydroxylated epimer **22**. The downfield shifts of the β carbon, namely, C-23, fell within the magnitude usually observed in analogous aliphatic alcohol series (5–12 ppm) (Table 4). Surprisingly, the C-20 signal

TABLE 4. Differences in ^{13}C Chemical Shifts of Adjacent Carbons Between Compounds with and without OH Substituent at C-22.[a]

Carbon	Compounds				
	19–2	**18–1**	**22–9**[b]	**23–8**	**17–4**
C-16	0.37	0.32	−0.51	−0.19	0.26
C-17	0.72	0.63	−3.72	0.30	0.52
C-20	−2.17	−2.23	5.13	−2.66	−2.39
C-21	1.31	1.25	−5.45	1.01	1.24
C-22	32.71	32.63	37.57	31.46	32.40
C-23	17.58	17.51	9.38	18.05	17.82
C-24	−0.23	−0.33	−3.35	−1.60	−0.33
C-25	1.34	1.35	1.95	1.57	1.54
C-26	−1.79	−1.74	−0.03	−2.54	−0.91

[a]Table entries are shift differences (Δδ).
[b]Compound **22** had β-OH at C-22.

moved upfield by ~2.5 ppm upon 22α hydroxylation to δ 33.5 and downfield shifted by ~5 ppm to δ 40.7 upon 22β hydroxylation. The reason for the perturbation on two β carbons in different directions due to C-22 hydroxylation is not immediately obvious. We also observed that C-21 and C-25 signals were slightly downfield shifted by ~1.5 ppm while C-26 moved slightly upfield by 1–2.5 ppm (Table 4). The C-22 acetylated triterpenes in this ^{13}C-nmr correlation study were all β-substituted. Acetylation of 22β-OH, which introduced a 2.8 ppm downfield shift of C-22, caused an unequal shielding on its β carbons. The signal for C-23 was shifted upfield by ~3.2 ppm while C-20 was shifted upfield by ~1.6 ppm. The effect of C-22β acetylation on C-24 was also observed (~−2.6 ppm). However, the effect on C-21 was not significant (<0.5 ppm) (Table 5) (7,8).

Functionalization of C-23 as an oxo group also resulted in a characteristic change to the signals corresponding to the carbons of the side chain. The C-23 oxo carbon resonated at 201.5 ppm, with concomitant upfield shifts of C-24 (~−11 ppm), C-20 (~−3 ppm), and C-26 (~−1.5 ppm); however, C-22, C-25, and C-27 were deshielded by ~17, 12.5, and 2.2 ppm, respectively (Table 6). This also confirmed that the 23-oxo group was cis to the C-27 methyl group. The opposite effect of the 23-oxo functionality on C-22 and C-24 was most likely due to an inductive effect on C-22 and a

TABLE 5. Differences in ^{13}C Chemical Shifts of Adjacent Carbons Between Compounds with and without OAc Substituent at C-22β.[a]

Carbon	Compounds					
	10–4	**11–5**	**12–1**	**13–2**	**20–9**	**21–8**
C-16	0.72	−0.30	−0.47	−0.21	−0.04	−0.21
C-17	−3.36	−3.37	−3.43	−3.37	−3.43	−3.39
C-20	3.39	3.69	3.63	3.76	3.52	3.36
C-21	−5.47	−5.52	−5.48	−5.40	−5.32	−5.48
C-22	39.89	39.82	40.00	39.88	40.40	39.77
C-23	5.91	5.99	5.98	6.10	6.15	6.00
C-24	−5.98	−6.00	−7.37	−5.87	−6.03	−6.02
C-25	2.43	2.43	3.55	2.45	2.85	2.12
C-26	−0.78	−1.33	−2.02	−1.73	−0.55	−1.84

[a]Table entries are shift differences (Δδ).

TABLE 6. Differences in ^{13}C Chemical Shifts of Adjacent Carbons Between Compounds with and without Oxo Substituent at C-23.[a]

Carbon	Compounds		
	14–3	**15–1**	**16–4**
C-20	−3.05	−3.10	−2.86
C-21	1.31	1.22	1.40
C-22	16.96	17.00	17.26
C-23	175.74	175.62	176.00
C-24	−11.17	−11.07	−11.04
C-25	12.84	12.64	12.57
C-26	−1.31	−1.10	−1.87
C-27	2.21	2.08	2.20

[a]Table entries are shift differences (Δδ).

combined polar resonance contribution of an α,β-unsaturated carbonyl system on C-24.

We also found that in all lanosta-7,9(11),24-trien-26-oic acids the C-27 methyl was most shielded and resonated at about 12 ppm. The chemical shift assignment was based on a strong correlation in the ^{13}C, ^{1}H heteronuclear 2D-nmr.

EXPERIMENTAL

CULTURE OF *G. LUCIDUM*.—*G. lucidum* of the strain TP-1 was collected locally and deposited at the Institute of Botany, Academia Sinica, Republic of China. This strain was maintained on potato-dextrose agar slants. For mycelial growth, fungi were inoculated in 1-liter culture flasks (× 30) containing 300 ml sterilized medium, which consisted of 20 g dextrose and 30 g malt extract per liter of distilled H_2O. Cultures were maintained stationary at 28 ± 1.5° for 30 days.

ISOLATION AND PURIFICATION.—Mycelia were harvested from a 30-day-old liquid culture of *G. lucidum*. After filtration through four layers of cheesecloth, the dried biomass was ground into powder and extracted with MeOH. The concentrated extracts were partitioned between *n*-hexane and H_2O. The aqueous layer was reextracted with EtOAc. The pooled EtOAc fraction was chromatographed on a Si gel column (45 × 2.5 cm) by stepwise elution with increasing percentage of MeOH in $CHCl_3$. The isolation and purification procedures for compounds **1–24** have been described previously (7–11).

GENERAL PROCEDURES IN NMR EXPERIMENTS.—^{1}H and ^{13}C-nmr spectra were taken with Bruker AM-400, AC-300, or MSL-200 spectrometers, and spectral data were reported as ppm downfield from TMS (δ = 0). Unless specified, samples were dissolved in $CDCl_3$ and spectra were taken at ambient temperature. For ^{13}C assignment, the broad-band decoupled ^{13}C and DEPT experiments were carried out for each compound. To make unambiguous assignment of certain carbon signals additional ^{1}H-^{1}H and ^{1}H-^{13}C shift correlated 2D-nmr experiments were also performed for some of the compounds.

ACKNOWLEDGMENTS

We thank the National Science Council (grant No. 78-0412-B075-03 to M.-S. Shiao) and Veterans General Hospital, Republic of China, for financial support. We also thank Prof. David E. Cane, Department of Chemistry, Brown University for his kind suggestions.

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Received 5 December 1988