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Total Assignment of ^{13}C and ^{1}H Spectra of Three Isomeric Triterpenol Derivatives by 2D NMR: An Investigation of the Potential Utility of ^{1}H Chemical Shifts in Structural Investigations of Complex Natural Products

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ABSTRACT

It is shown that a recently proposed indirect heteronuclear shift-correlated pulse sequence, XCORFE, can be used to unambiguously assign the ^{13}C spectra of three isomeric ($C_{30}H_{50}O$) triterpenols: taraxasterol (1a), pseudo-taraxasterol (2) and lupeol (3). This sequence gives excellent resolution combined with sensitivity far in excess of that given by ^{13}C-^{13}C connectivity experiments. Direct heteronuclear shift-correlated spectra are used to totally assign ^{1}H spectra for 1a, 2, 3 and taraxasteryl acetate (1b). ^{1}H chemical shifts are mainly sensitive to local environment and often show values which are characteristic of a particular environment. Knowledge of ^{1}H chemical shifts and splitting patterns also places useful constraints on assignment of ^{13}C chemical shifts for $^{13}C^{1}H_{n}$ units. It is strongly recommended that natural products chemists routinely use 2D NMR to assign ^{1}H chemical shifts of complex organic derivatives in order to build up a data bank of ^{1}H spectral data.

Early in the development of chemical applications of NMR spectroscopy, it became apparent that methyl ^{1}H chemical shifts for steroids and terpenoids provided useful information concerning conformations and configurations of these complex organic derivatives.[1-3] In principle, additional information should be provided by methylene and methine ^{1}H chemical shifts. However, even with modern high field spectrometers, ^{1}H spectra are generally much too complex to allow direct assignment of methylene and methine chemical shifts. In the specific case of triterpene derivatives, even the assignment of methyl ^{1}H singlets is non-trivial and it has been necessary to resort to the use of shift reagents as an aid to assignment.[4]

However, the recent development of two-dimensional NMR spectroscopy[5] has provided a number of new NMR assignment techniques which are useful in the area of natural products chemistry.[6] In particular, the direct heteronuclear (^{1}H-^{13}C) chemical shift-correlated spectra allow simultaneous determination of ^{1}H and ^{13}C chemical shifts for directly bonded $^{13}C^{1}H_{n}$ units.[7] Provided that one has secure ^{13}C chemical shift assignments, it is thus feasible to assign totally ^{1}H spectra of organic derivatives by taking advantage of the better ^{13}C spectral resolution.[5,6]

In this paper we assess the potential utility of ^{1}H chemical shifts as a tool for structural investigations of steroids and terpenoids by totally assigning ^{13}C and ^{1}H chemical shifts for three isomeric ($C_{30}H_{50}O$) triterpenol derivatives plus ^{1}H chemical shifts for the acetate of one of these terpenols. We also demonstrate that a recently proposed indirect ^{13}C-^{1}H shift-correlated pulse sequence, XCORFE (X-nucleus correlation with fixed evolution time),[8] is very useful for unambiguously assigning ^{13}C spectra of these derivatives.

RESULTS AND DISCUSSION

The four compounds chosen for investigation were taraxasterol (18α,19α-urs-20(30)-en-3β-ol), 1a, plus its acetate, 1b, pseudotaraxasterol (18α,19α-urs-20-en-3β-ol), 2, and lupeol (lup-20(29)-en-3β-ol), 3:

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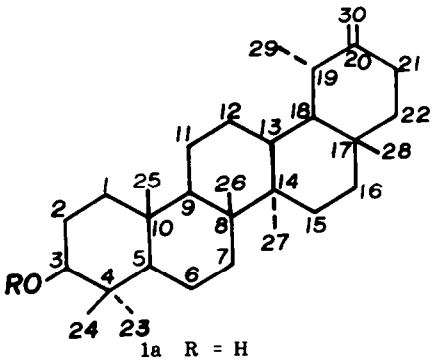
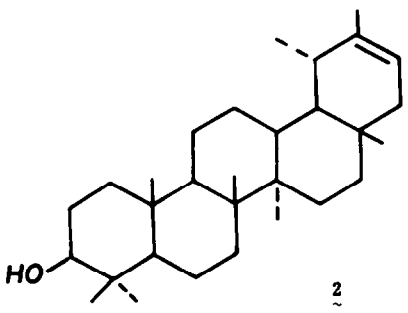


1a R = H
1b R = $COCH_3$



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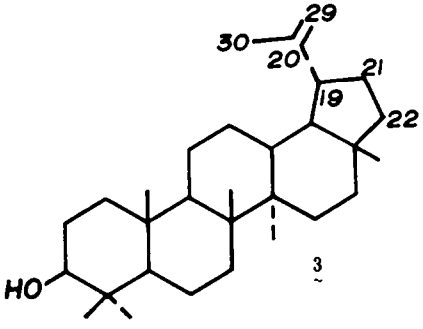

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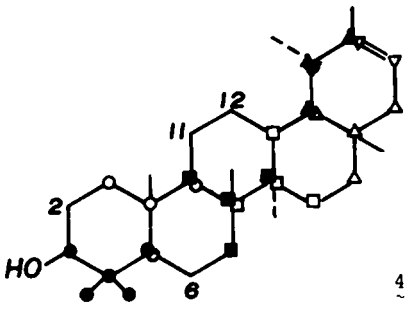
It was felt that this series of compounds would provide a useful test of the sensitivity of ^{1}H chemical shifts throughout the molecule to changes in structure in ring E as well as to the effect of making a minor alteration of a functional group.

a. *Assignment of ^{13}C chemical shifts for 1a, 2 and 3*

An essential prerequisite to unambiguous assignment of ^{1}H chemical shifts from ^{13}C–^{1}H shift-correlated spectra is unambiguous assignment of ^{13}C chemical shifts of protonated carbons. This is a non-trivial problem, as illustrated by our recent publication[9] which demonstrated that 10 of the 24 protonated carbons in 1b had been misassigned in an earlier investigation.[10] In that case, we used a two-dimensional ^{13}C–^{13}C connectivity experiment[11] to assign the ^{13}C spectrum. While this experiment gives unambiguous results, it suffers from extremely low sensitivity.[5] Fortunately, higher sensitivity experiments are available which can often be used in combination to arrive at unambiguous assignments.

We initially used normal and DEPT-edited[12] spectra to partially assign ^{13}C spectra in terms of numbers of attached protons. The results of these experiments were consistent with earlier assignments for 3,[13] but indicated errors in reported assignments for 1a.[10] No previous ^{13}C spectral assignments were available for 2, although the spectrum of its acetate had been assigned.[14] However, several of these assignments were uncertain[14] and others seemed inconsistent with our edited spectra for 2. Consequently, we decided to determine indirect heteronuclear shift-correlated spectra (i.e., with delay times optimized for two- and three-bond ^{13}C–^{1}H couplings)[7,15] for these compounds as an aid to spectral assignment. The methyl ^{1}H signals in 1a, 2 and 3 are ideal for this purpose. Typically, $^{2}J_{CH} \cong {}^{3}J_{CH} \cong 4$–5 Hz for methyl protons,[16] allowing simultaneous optimization of delay times for both two- and three-bond connectivities. Methyl groups also give sharp, intense ^{1}H peaks, optimizing both the resolution and sensitivity of the experiment. Finally, methyl groups are strategically located in these triterpenols, providing a network of two- and three-bond connectivities which tie the molecule together and allow assignment of the ^{13}C spectrum. This is illustrated in 4 for pseudotaraxasterol.

Indirect shift-correlated experiments were carried out for 1a, 2 and 3 using the recently proposed pulse sequence, XCORFE.[8] This is one of a family of related sequences[8,17,18] which are



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Connectivities

- ● H-23, H-24
- ○ H-25
- ■ H-26
- □ H-27
- △ H-28
- ▲ H-29
- ▽ H-30

designed to increase sensitivity by incorporating the variable time t_1, used to establish ^{1}H frequencies, inside the fixed delay T, used to set up ^{1}H to ^{13}C polarization transfer. This minimizes signal loss due to ^{1}H T_2 relaxation prior to polarization transfer.[17] The delay prior to polarization transfer was set at 0.109 s and that after polarization transfer at 0.04 s, both optimized for methyl protons with $J_{CH} \approx 4.5$ Hz.[5]

Some typical cross-sections through ^{13}C frequencies of 2 are illustrated in Figure 1. The starting point for assignment was the gem-dimethyl groups. These provide the only examples where one observes a cross-peak corresponding to a methyl ^{1}H signal and a ^{13}C signal from a second methyl group (H-23 with C-24 (Figure 1a) and H-24 with C-23 (Figure 1b)), allowing identification of H-23 and H-24. H-23 and H-24 also show cross-peaks at ^{13}C frequencies corresponding to C-3, C-4 and C-5. At one of these (C-5) there is a third signal which must correspond to H-25 (Figure 1C). Similarly, one of the methine carbons (C-9) shows a cross-peak from H-25 and a second methyl signal which must correspond to H-26 (Figure 1d). Working along the molecule in this fashion, it is possible to assign all methyl ^{1}H signals. Similarly, most non-protonated, methine and methylene carbons can be assigned from cross-peaks with specific methyl protons and from their known numbers of attached protons from DEPT spectra. For example, C-3, C-4 and C-5 all show cross peaks with H-23 and H-24. C-4 is readily assigned as the only quaternary carbon of the three, while C-3 and C-5 are distinguished by the additional H-25 cross peak for the latter carbon. Observed connectivities and assigned carbons for 2 are summarized in Table 1.

Four methylene carbons (C-2, C-6, C-11 and C-12) showed no cross-peaks with methyl protons, as expected (see 4). Fortunately, these carbons all showed cross-peaks with adjacent methylene or methine protons.[19] One advantage of XCORFE is that it often allows distinction between two-bond (^{1}H-C-^{13}C) and three-bond (^{1}H-C-C-^{13}C) connectivities since the peak corresponding to a two-bond connectivity shows coupling (in F_1) to any protons directly bonded to ^{13}C.[8] This is illustrated in Figure 1e which shows a two-bond connectivity between H-5 and C-6, while Figure 1f shows a three-bond connectivity between H-5 and C-25, and a similar weak connectivity between H-5 and C-24 is seen in Figure 1a.[20] C-1, C-5, C-9 and C-13 could all be unambiguously assigned from connectivities to methyl ^{1}H signals while their attached protons could be assigned from a direct heteronuclear shift-correlated experiment (see Part b). In turn, connectivities of these protons to C-2, C-6, C-11 and C-12 (see Table 1) allowed unambiguous assignment of these methylene carbons. C-16 and C-22 could not be distinguished from the data in Table 1 since both are methylene carbons with cross peaks only from H-28. However, they could readily be distinguished from their proton multiplet patterns in a standard heteronuclear shift-correlated experiment (Part b). Finally, all methyl ^{13}C signals were assigned from their direct connectivities with previously assigned methyl ^{1}H signals. Only C(8) and C(14) provided no basis for assignment from the shift-correlated experiments. However, these quaternary carbons can be assigned with reasonable confidence from the earlier assignment of 1b[9] since the chemical shift of C-8 in particular is almost invarient for 1a, 1b, 2 and 3.

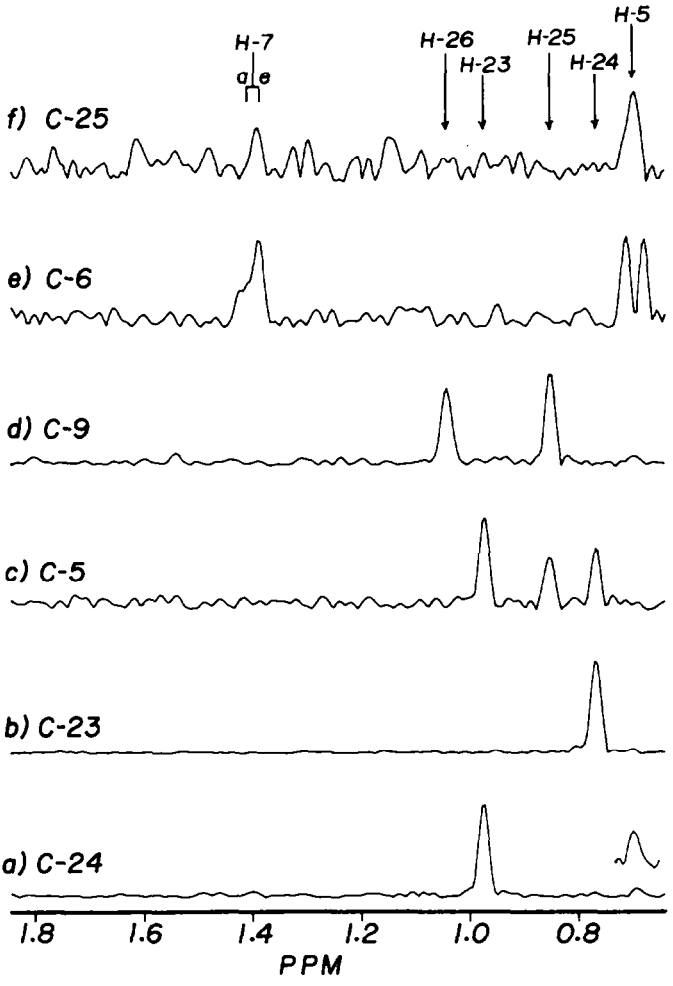

FIGURE 1. Cross-sectional plots through specific ^{13}C frequencies from the indirect ^{13}C–^{1}H shift-correlated spectrum of 2 obtained with the pulse sequence XCORFE. ^{1}H assignments are indicated at the top while ^{13}C assignments are indicated on each plot. In addition to the cross-peaks discussed in the text, C-6 also shows cross-peaks with H-7_a and H-7_e. The expected doublet splitting of the former signal is apparently obscured by partial overlap with H-7_e.

TABLE 1. Observed $^{1}H-^{13}C$ Cross Peaks in Indirect Shift-Correlated Spectrum for 2 Plus Assigned ^{13}C Peaks

δ_C [a]	n_H [b]	Observed ^{1}H connectivities	Carbon
139.81	0	H-29, H-30	C(20)
118.89	1	H-30	C(21)
78.98	1	H-23, H-24	C(3)
55.31	1	H-23, H-24, H-25	C(5)
50.43	1	H-25, H-26	C(9)
48.72	1	H-28, H-29	C(18)
42.34	0	H-26, H-27	C(14)
42.18	2	H-28	C(22)
41.08	0	H-26, H-27	C(8)
39.23	1	H-27	C(13)
38.86	0	H-23, H-24	C(4)
38.77	2	H-25	C(1)
37.10	0	H-25	C(10)
36.71	2	H-28	C(16)
36.32	1	H-29 (d, J ~ 8 Hz),[c] H-30	C(19)
34.39	0	H-28	C(17)
34,25	2	H-26	C(7)
28.00	3	H-24	C(23)
27.64	2	H-13 (d, J ~ 12 Hz)	C(12)
27.41	2	$H-1_e$	C(2)
27.04	2	H-27	C(15)
22.54	3	--	C(29)
21.66	3	--	C(30)
21.56	2	H-9 (d, J ~ 12 Hz)	C(11)
18.31	2	H-5 (d, J ~ 12 Hz), $H-7_a$, $H-7_e$	C(6)
17.71	3	H-19 (q, J ~ 8 Hz)	C(28)
16.30	3	H-5	C(25)
16.06	3	--	C(26)
15.40	3	H-23, H-5	C(24)
14.75	3	--	C(27)

[a] ^{13}C chemical shifts relative to internal $Si(CH_3)_4$.

[b] Number of attached protons as determined from normal and DEPT-editted ^{13}C spectra.

[c] Value in parentheses indicates observed H-H coupling and multiplicity (d = doublet, q = quartet). With XCORFE, peaks corresponding to two-bond $^{1}H-^{12}C-^{13}C$ connectivities are split by $^{1}H-C-^{13}C-^{1}H$ coupling, when present (see ref. 9). All other peaks appear as singlets.

Similar indirect shift-correlated spectra were used to assign the spectra of 1a and 3. Assignments are given in Table 2. The assignments for 3 are identical with those previously proposed by Wenkert et al.[13] but there are several errors in the previously reported assignments for 1a.[10]

TABLE 2. Assigned ^{13}C Peaks for 1a and 3 [a]

	δ_C [b]			δ_C [b]	
Carbon	1a	3	Carbon	1a	3
C(1)	38.77	38.67	C(16)	38.31	35.54
C(2)	27.35	27.35	C(17)	34.53	42.95
C(3)	79.01	78.94	C(18)	48.67	48.24
C(4)	38.82	38.81	C(19)	39.40	47.94
C(5)	55.37	55.25	C(20)	154.57	150.88
C(6)	18.29	18.28	C(21)	25.62	29.80
C(7)	34.06	34.23	C(22)	38.88	39.96
C(8)	40.86	40.78	C(23)	28.02	27.95
C(9)	50.48	50.38	C(24)	15.39	15.35
C(10)	37.12	37.11	C(25)	16.78	16.09
C(11)	21.43	20.89	C(26)	15.87	15.94
C(12)	26.17	25.08	C(27)	14.76	14.51
C(13)	39.17	38.00	C(28)	19.49	17.97
C(14)	42.02	42.78	C(29)	25.54	109.31
C(15)	26.64	27.41	C(30)	107.15	19.28

[a] ^{13}C chemical shifts for 1b were also redetermined but these are not listed since they are identical (within 0.05 PPM) with those previously reported (see ref. 9).

[b] ^{13}C chemical shifts in PPM relative to $(CH_3)_4Si$.

The results in Figure 1 and Tables 1 and 2 clearly demonstrate the usefulness of XCORFE for assigning ^{13}C spectra. The sensitivity of this sequence is particularly impressive. The spectra in Figure 1 were obtained for 50 mg of sample with a total measuring time of 2 h. The signal-to-noise ratio is sufficiently high that unambiguous results for methyl ^1H cross-peaks could have been obtained in one-quarter of that time and/or with significantly less sample. Spectra were obtained using a spectrometer operating at 400 MHz for ^1H with a 5 mm multi-nuclear probe. By contrast, our earlier ^{13}C–^{13}C connectivity spectra (obtained on a 300 MHz spectrometer with a 10 mm probe) required 36 h for 300 mg of sample.[8] Even if carried out with a spectrometer identical to that used in this experiment, the ^{13}C–^{13}C connectivity experiment would require at least 100 mg of sample for an overnight run.[21] XCORFE also has advantages over Kessler's COLOC sequence.[17] Specifically, the elimination of spurious peaks due to direct ^1H–^{13}C connectivities simplifies spectral interpretation while the ability of XCORFE to distinguish two- and three-bond connectivities (via vicinal ^1H–^1H coupling)[8] was particularly useful in completing the assignment of carbons which show no methyl ^1H cross peaks.

Bax et al. have recently proposed the use of the INEPT pulse sequence with selective 180° ^1H refocusing pulses (INAPT[22]) as an approach to establishing indirect ^1H–^{13}C connectivities. While this should be even more sensitive than XCORFE, it has the disadvantage that the ^1H peaks which are selectively excited must be separated by at least 10 Hz (0.025 PPM at 400 MHz).[22] By contrast, using XCORFE, it was possible to distinguish ^1H peaks separated as little as 0.01 PPM. Thus XCORFE provides both very good sensitivity and excellent resolution.

b. *Assignment of ^{1}H chemical shifts for 1a, 1b, 2 and 3*

Methine and methylene proton signals were assigned from direct connectivities determined using the heteronuclear shift-correlated pulse sequence optimized for one-bond ^{1}H–^{13}C couplings. ^{1}H chemical shifts were determined using the version of this sequence which gives partial (all except geminal) ^{1}H–^{1}H decoupling.[23] This was chosen to give best sensitivity and most precise measurement of ^{1}H–^{1}H chemical shifts. A typical contour plot is shown in Figure 2. Note that although there are 31 distinct proton signals in the region δ 0.7–1.8, these are generally well resolved due to the ^{13}C spectral dispersion. A second set of spectra was obtained using the standard heteronuclear shift-correlated sequence[7] which revealed proton coupling patterns. F_1 ^{1}H spectral width divided by the number of time increments was 7–8 Hz, allowing resolution of ^{1}H–^{1}H couplings larger than this magnitude. The splitting patterns reveal the number of large (*gauche* or *anti*-vicinal) couplings experienced by each proton, allowing assignment of protons as axial or equatorial (ring E protons could not be assigned in this way since this ring is not in a chair conformation[9]). ^{1}H chemical shifts, coupling patterns and conformational assignments are summarized in Table 3.

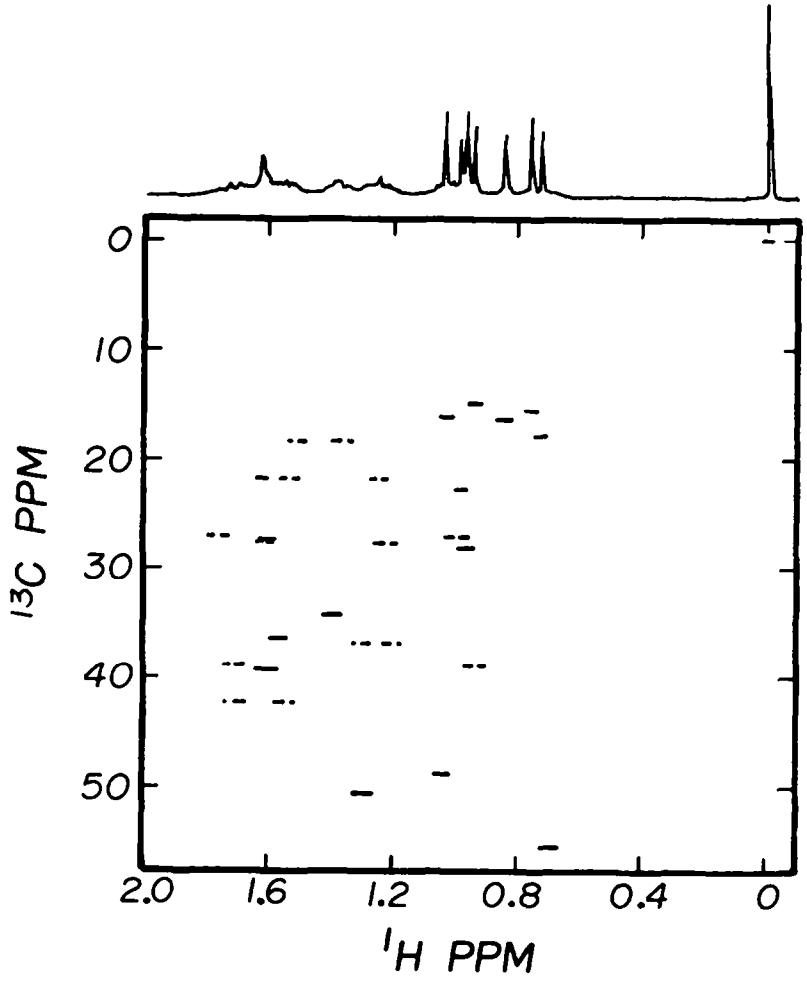

FIGURE 2. Contour plot of the direct ^{13}C–^{1}H shift-correlated spectrum of 2 illustrating the saturated carbon region. Note the complete lack of parallel between ^{1}H and ^{13}C chemical shifts.

TABLE 3. ^{1}H Chemical Shifts for 1a, 1b, 2 and 3

Hydrogen	δ_H[a] 1a	1b	2	3
1	0.95(t)$_a$, 1.73(d)$_e$	1.03$_5$(t)$_a$, 1.72(d)$_e$	0.95(t)$_a$, 1.73(d)$_c$	0.91(t)$_a$, 1.68$_5$(d)$_e$
2	1.59(q)$_a$, 1.63(d)$_e$	{1.66}(m)[b]	1.58(q)$_a$, 1.64(d)$_e$	1.54(q)$_a$, 1.61(d)$_e$
3	3.20(dd)$_a$[c]	4.48$_5$(dd)$_a$[c]	3.19(dd)$_a$[c]	3.18$_5$(dd)[c]
5	0.70(d)$_a$	0.80(d)$_a$	0.70(d)$_a$	0.69(d)$_a$
6	1.38$_5$(q)$_a$, 1.53(d)$_e$	1.39(q)$_a$, 1.51(d)$_e$	1.38$_5$(q)$_a$, 1.52(d)$_e$	1.39(q)$_a$, 1.54(d)$_e$
7	1.35(m), 1.39(m)	{1.39}(m)[b]	1.37(m), 1.41(m)	{1.41}(m)[b]
9	1.33(d)$_a$	1.34(d)$_a$	1.30$_5$(d)$_a$	1.28(d)$_a$
11	1.28(q)$_a$, 1.54(d)$_e$	1.29(q)$_a$, 1.54(d)$_e$	1.26(q)$_a$, 1.58(d)$_e$	1.25(q)$_a$, 1.42(d)$_e$
12	1.10(q)$_a$, 1.69(d)$_e$	1.10(q)$_a$, 1.68(d)$_e$	1.23(q)$_a$, 1.62(d)$_e$	1.07(q)$_a$, 1.68(d)$_e$
13	1.60(t)$_a$	1.59(t)$_a$	1.61(t)$_a$	1.67(t)$_a$
15	0.96(d)$_e$, 1.68(t)$_a$	0.94(d)$_e$, 1.68(t)$_e$	1.01(d)$_e$, 1.78(t)$_a$	1.01(d)$_e$, 1.71(t)$_a$
16	1.16(t)$_a$, 1.25(d)$_e$	1.15(t)$_a$, 1.23$_5$(d)$_e$	1.21(t)$_a$, 1.31(d)$_e$	1.38(t)$_a$, 1.49(d)$_e$
18	0.97(t)$_a$	0.96$_5$(t)$_a$	1.04$_5$(t)$_a$	1.37(t)$_a$
19	2.11(m)	2.10(m)	1.57(m)	2.39(m)
21	2.20(m), 2.45(m)	2.21$_5$(m), 2.45$_5$(m)	5.25(d)[b]	1.33(m), 1.93(m)
22	1.37(m), 1.41(m)	1.36(m), 1.42(m)	1.56(d), 1.72(d)	1.20$_5$(m), 1.42(m)
23	0.97(s)	0.85$_5$(s)	0.98(s)	0.98$_5$(s)
24	0.76(s)	0.84$_5$(s)	0.78(s)	0.77(s)
25	0.85(s)	0.86$_5$(s)	0.86(s)	0.84(s)
26	1.02$_5$(s)	1.025(s)	1.04$_5$(s)	1.04(s)
27	0.94(s)	0.93(s)	0.95$_5$(s)	0.97(s)
28	0.86$_5$(s)	0.85(s)	0.73(s)	0.79(s)
29	1.02(d)	1.02(d)	0.99(d)	4.56(m), 4.69(m)[c]
30	{4.61}(m)[c]	{4.61}(m)[c]	1.64(s)	1.69(s)
OAc	--	2.05(s)	--	--

[a] ^{1}H chemical shifts relative to internal Si(CH$_3$)$_4$. The letter in parentheses indicates the observed splitting pattern: (s) singlet, (d) doublet, (t) triplet, (q) quartet and (m) unresolved multiplet. Subscript a or e indicates an axial or equatorial hydrogen where this can be distinguished from the splitting pattern.

[b] Chemical shift difference between methylene protons is too small to allow determination of individual chemical shifts. It is estimated that individual chemical shifts differ from average value by less than 0.02 PPM.

[c] ^{1}H chemical shifts and splitting patterns determined directly from ^{1}H spectrum.

These data indicate that ^{1}H chemical shifts are mainly sensitive to local structural change with smaller effects at intermediate range and normally negligible effects at long range. This is particularly apparent for 1a and 1b which differ only in chemical shifts for ring A protons and methyl groups attached to ring A. In the case of 1a, 2 and 3, chemical shifts for rings A and B are almost insensitive to variations in ring E structure (although 3 does show variations of up to 0.05 PPM for H-1 and H-2). There are minor variations for H-11 and H-12 in ring C, likely reflecting strong steric interactions between H-12 and H-29,[9] while there are significant variations for H-16 in ring D. In ring E, C-18, C-19 and C-22 are the only protonated carbons which are saturated in all three isomers. Their protons show major variations with structure. The signal for the attached methyl group, H-28, also shows significant dependence on structure.

The data in Table 3 indicate that there are characteristic chemical shifts associated with specific local environments. For example, H-5 occurs at δ 0.7 in all three triterpenols. This unusual chemical shift may reflect the effect of the adjacent gem-dimethyl group. As another example, the allylic protons H-19 in 3 and H-19 and H-21 in 1a all give shifts in the range δ 2.1 - 2.5, while in 2, H-19 and H-22 (which are allylic to the endocyclic double bond) show chemical shifts in the range δ 1.56 - 1.72. Thus there is an obvious and significant difference between the effects of endo- and exocyclic double bonds. Even more important from the point of view of ^{13}C assignments is the observation that while both ^{13}C and ^{1}H chemical shifts are characteristic of their specific environment, there is no simple relationship between ^{13}C and ^{1}H chemical shifts for individual CH_n groups. This can be clearly seen in Figure 2 which shows an apparently random relationship for the two chemical shift parameters. Thus, in cases where two protonated carbons have very similar chemical shifts, it may still be trivial to assign them if the chemical shifts of their attached protons are significantly different and/or if they show different ^{1}H–^{1}H splitting patterns. For example, in the original investigation of 1b, C(21) (δ 25.61) was incorrectly assigned as C(12) (δ 26.15).[10] However, with the shift-correlated experiment, the correct assignment of C(21) is trivial due to the characteristic allylic shifts of its protons (δ 2.21, 2.46, compared to δ 1.10, 1.69 for the methylene protons of C(12)). Furthermore, C(12) is also easily distinguished from C(15) (δ 26.64) since $H\text{-}15_a$ shows only one *anti*-vicinal coupling compared to two for $H\text{-}12_a$ (see Table 3). Similarly, in the case of 2, C(16) (δ 36.7) and C(22) (δ 42.2) are readily distinguished since the H-22 protons show no large couplings other than geminal couplings (Table 3).

CONCLUSIONS

The indirect shift-correlated pulse sequence XCORFE[7] is particularly useful for assigning ^{13}C spectra of complex terpenoids while the direct shift-correlated spectra allow total assignment of ^{1}H chemical shifts. The data obtained from the latter experiment are useful not only for structural assignment but also because they place significant and useful constraints on the assignment of ^{13}C chemical shifts.

We would strongly recommend that workers in the field of natural products chemistry should routinely use the direct heteronuclear shift-correlated sequence to determine ^{1}H chemical shifts, not only for new compounds but also for any known compounds for which complete ^{1}H chemical shift assignments have not been previously made. While it would also be desirable to use either ^{13}C–^{13}C connectivity experiments[11] or indirect shift-correlated experiments[8,15,17,18] to assign unambiguously ^{13}C chemical shifts, this may not always be essential due to the constraints placed on ^{13}C chemical shift assignments by the ^{1}H spectral data. As the ^{1}H spectral data bank is built up, it should be increasingly possible to use these data for detailed structural assignments. In fact, the combination of ^{1}H chemical shift and ^{1}H–^{1}H coupling information along with ^{13}C chemical shifts is probably ideally suited for incorporation into computer programs for structural assignment from spectral data.

EXPERIMENTAL

Details of isolation of 1a and 1b have been reported previously.[9] Compound 2 (mp 217-219°) was synthesized from 1b by refluxing in benzene:ethanol containing 10% H_2SO_4.[24] Compound 3 (mp 215-216°) was isolated from *Clathrotropis brachypetala* during the course of a systematic phytochemical investigation of this plant. Full details will be reported elsewhere.[25]

All ^{13}C, ^{1}H and two-dimensional spectra were obtained on a Varian XL-400 spectrometer equipped with a 5-mm multinuclear probe (probe temperature 18°, ^{13}C 90° pulse width = 13 μs, ^{1}H decoupler 90° pulse width = 27 μs). Each sample contained 50 mg of triterpenoid derivative in *ca.* 0.5 mL of $CDCl_3$. The spectra in Figure 1 were obtained with an f_1 (^{1}H) spectral width of 440 Hz with 96 time increments, zero filled to 512. The F_2 (^{13}C) width was 14,500 Hz with 2048 data points, zero filled to 4096. Sixty-four transients were collected for each time increment with

a relaxation delay of 1.0 s. Total acquisition time was 2.1 h. The spectrum in Figure 2 was obtained using an f_1 spectral width of 840 Hz with 128 time increments (zero filled to 512), an f_2 spectral width of 6000 Hz with 1024 data points (zero filled to 2048), 32 transients per time increment and a relaxation delay of 0.66 s. Total acquisition time was 1.0 h. Similar experimental parameters were used for obtaining the two-dimensional spectra for 1a, 1b and 3. In the case of XCORFE spectra, pseudo-echo processing[26] was used in the t_1 dimension, while modified pseudo-echo processing[27] was used in the t_2 dimension. Modified pseudo-echo processing was used in both t_1 and t_2 for the other two-dimensional spectra. ^1H chemical shifts reported in Table 3 were determined from cross-sectional plots through ^{13}C frequencies (similar to those in Figure 1). Chemical shifts are believed to be accurate to within 0.02 PPM.

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19. In addition to the obvious difference that they involve polarization transfer from a single proton rather than from three protons in the case of a methyl group, these cross-peaks tend to be of much lower sensitivity due to more rapid ^1H T_2 relaxation for methine and methylene protons than methyl protons, due to their wider range of two- and three-bond ^{13}C–^1H coupling (see ref. 16) and due to ^1H–^1H coupling vectors attaining a partial anti-phase orientation at the time of polarization transfer (see ref. 18).
20. The connectivity between H-5 and C-24 indicates an anti orientation of these atoms, confirming the assignment of the gem-dimethyl carbons. The corresponding gauche-vicinal coupling is expected to be much smaller (see ref. 16), resulting in a much weaker cross-peak between H-5 and C-23.
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