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APPLICATION OF INVERSE-DETECTED TWO-DIMENSIONAL HETERONUCLEAR-CORRELATED NMR SPECTROSCOPY TO THE COMPLETE CARBON-13 ASSIGNMENT OF ISOMULTIFLORENYL ACETATE

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ABSTRACT.—The ^{13}C-nmr assignment of a new pentacyclic triterpene, isomultiflorenyl acetate [**1**], found in *Benincasa cerifera*, has been resolved using inverse-detected two-dimensional heteronuclear-correlated nmr spectroscopy.

The wax gourd, *Benincasa cerifera* Savi (syn. *Benincasa hispida* Thunb.) (Cucurbitaceae), which grows throughout the Asian tropics, is characterized by a waxy coating of its fruit. Although the sterolic fraction of the fleshy part of this melon-like fruit has been investigated (1), the chemical composition of the "wax" has not been studied. We have isolated a pentacyclic triterpene from the terpenoid fraction, identified as isomultiflorenyl acetate [**1**], which has not been found before as a natural product (2). Scrutiny of the data used to establish the structure of pentacyclic triterpenes (3–6) reveals that, for most of them, uncertainties and ambiguities exist in the ^{1}H- and ^{13}C-nmr assignments.

In this paper we illustrate an nmr strategy that unambiguously solves the ^{13}C assignment of pentacyclic triterpenes using inverse-detected two-dimensional heteronuclear-correlated nmr spectroscopy.

RESULTS AND DISCUSSION

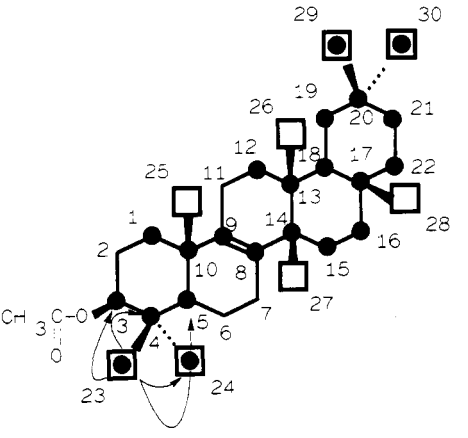
The ^{13}C-nmr spectrum of isomultiflorenyl acetate [**1**] consists of 32 resolved signals. Beyond confirming the presence of an acetate function, the multiplicities of the individual signals determined using the DEPT pulse sequence (7) indicated a tetrasubstituted double bond, six aliphatic quaternary carbons, eight methyl groups, and three methine and eleven methylene resonances.

Although the majority of these signals are found in an unresolved envelope, examination of the 400 MHz ^{1}H-nmr spectrum [δ 4.50 (1H, dd, J = 10.9, 5.1 Hz, C*H*O), 2.05 (3H, s, CH_3CO), 1.07 (3H, s), 1.05 (3H, s), 0.98 (6H, s), 0.97 (3H, s), 0.95 (3H, s), 0.88 (3H, s), 0.87 (3H, s)] indicated a hydrogen alpha to an oxygen function and eight methyl groups linked to aliphatic quaternary carbons. The presence of two *gem*-dimethyl groups was further supported by the number of sp^3-hybridized quaternary carbons.

From the above results a likely molecular formula for this compound ($C_{32}H_{52}O_2$) was verified by the molecular ion in the mass spectrum at *m/z* 468. At this point, compound **1** was identified as isomultiflorenyl acetate on the basis of the above arguments and general similarity of its ^{1}H-nmr spectrum with that of previously reported synthesized isomultiflorenyl acetate (6).

The relatively small quantity of material available (ca. 40 mg) precluded the use of the INADEQUATE double quantum 2D experiments (8,9). Since the quantity available was more than adequate for ^{1}H-nmr work, we have used inverse detection techniques to provide complete and unambiguous ^{13}C assignments. These techniques are

the ^1H detected one-bond (C,H) heteronuclear multiple quantum coherence (HMQC) (10) and the long range (two and three bonds) (C,H) heteronuclear multiple quantum bond connectivity (HMBC) (11) experiments. Because the ^1H spectrum shows severe overlap, assignments of CH_n groups were established using the long range correlation peaks between proton methyl shifts and carbons α and β to these groups (Figure 1).



1

FIGURE 1. Long range proton-carbon connectivities derived from the proton detected C,H correlation of isomultiforenyl acetate [**1**]. Protons and carbons interconnected are indicated respectively by squares and dots (long-range C,H connectivities are illustrated for Me-23).

One-bond proton-carbon chemical shift correlations were established using the proton-detected C,H correlation experiment providing the identities of the direct responses as shown in Figure 2. Carbon-hydrogen pairings of the methyl groups are readily determined from this HMQC contour plot (Table 1). Among the remaining spin systems, the deshielded proton resonances of the two methylene groups can be noted at 27.48 and 20.93 ppm in the ^{13}C-nmr spectrum. These signals can be ascribed, respectively, to carbons C-7 and C-11 alpha to the double bond function, using chemical shift arguments and revised assignments for lanosterol (12).

TABLE 1. Carbon-Proton Pairs for Methyl Groups of Isomultiflorenyl Acetate [**1**].[a]

^1H	^{13}C
1.07	31.59
1.05	24.95
0.98	18.93 and 20.00
0.97	33.22
0.95	34.70
0.88	28.12
0.87	16.77

[a]^1H and ^{13}C resonances for methyl acetate are not indicated.

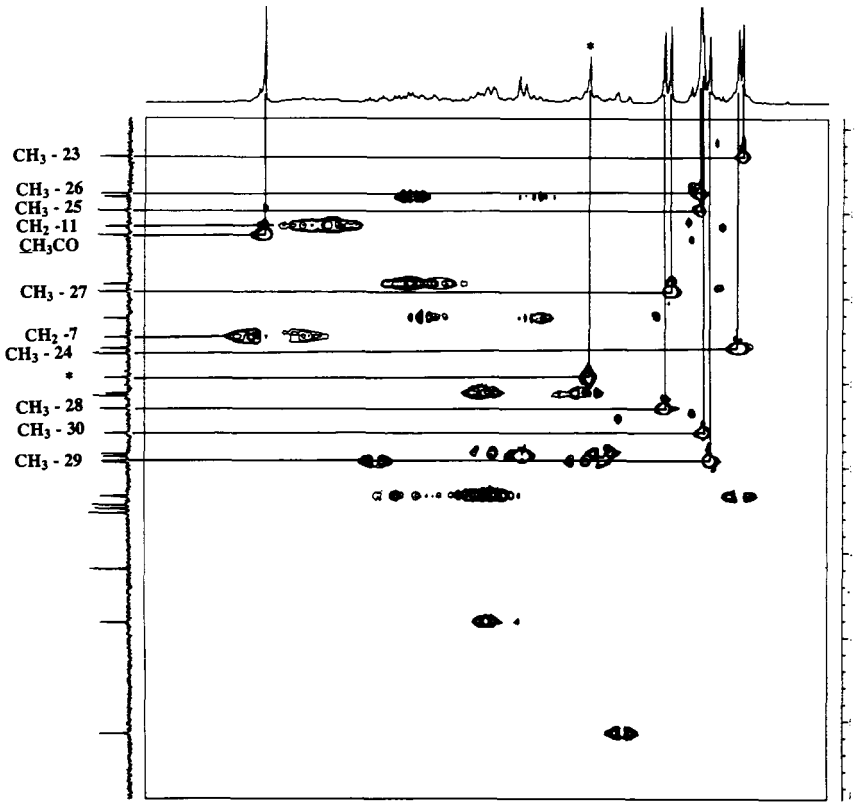


FIGURE 2. High field part of the HMQC contour plot for isomultiflorenyl acetate [**1**]. Signals indicated by an asterisk denote impurity.

TABLE 2. ^{13}C nmr Chemical Shifts and Assignments for Isomultiflorenyl Acetate [**1**].

Carbon	Group	^{13}C	Carbon	Group	^{13}C
C-1	CH_2	34.94	C-17	C	31.04
C-2	CH_2	24.39	C-18	CH	44.32
C-3	CH	81.11	C-19	CH_2	34.30
C-4	C	37.91	C-20	C	28.42
C-5	CH	51.05	C-21	CH_2	43.02
C-6	CH_2	19.29	C-22	CH_2	36.91[a]
C-7	CH_2	27.48	C-23	Me	16.77
C-8	C	135.43	C-24	Me	28.12
C-9	C	133.62	C-25	Me	20.00
C-10	C	37.67	C-26	Me	18.93
C-11	CH_2	20.93	C-27	Me	24.95
C-12	CH_2	31.04	C-28	Me	31.59
C-13	C	37.53	C-29	Me	34.70[b]
C-14	C	41.12	C-30	Me	33.22[b]
C-15	CH_2	26.56	$COCH_3$	C	170.90
C-16	CH_2	37.08[a]	$COCH_3$	Me	21.27

[a,b]Assignments may be reversed.

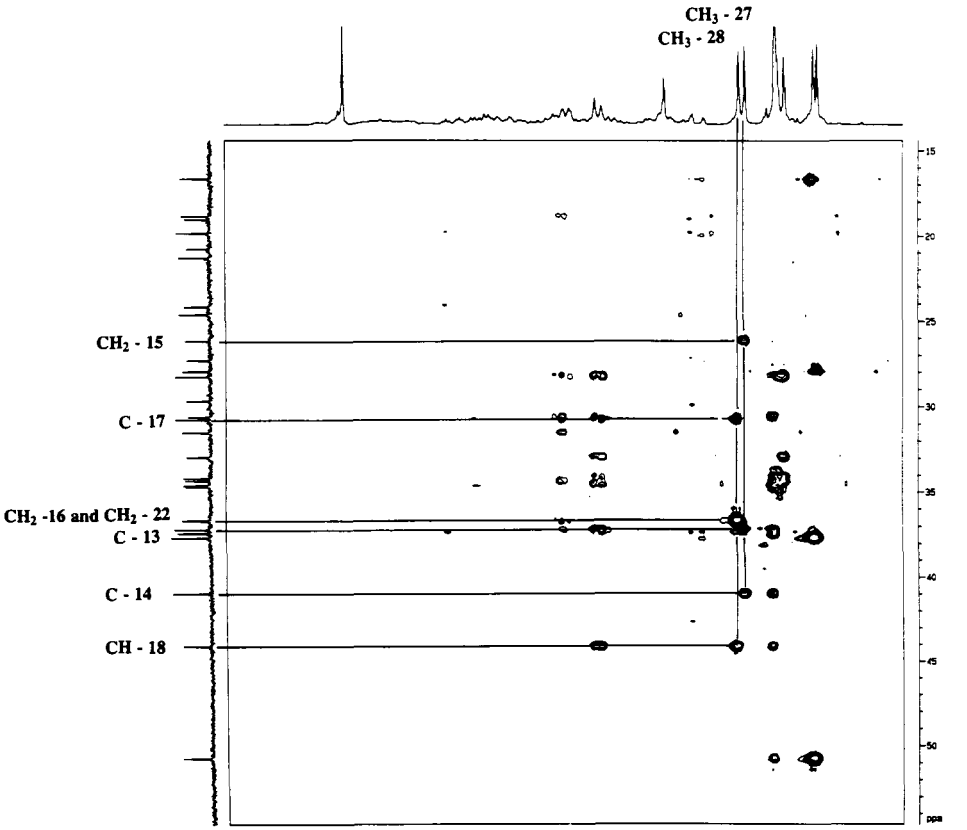
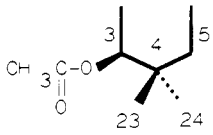


FIGURE 3. High field expanded region of the HMQC contour plot of isomultiflorenyl acetate [**1**]. Long-range connectivities are indicated for Me-28 and Me-27.

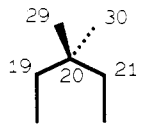
By utilizing the HMBC plot (Figure 3), the structural fragments **A–D** can be determined using the connectivities observed for the corresponding proton methyl shifts. Considering substructure **B**, the H-19 resonance can be identified with certainty in the ^{1}H-detected one-bond C,H heteronuclear multiple quantum coherence experiment (δ 1.45 ppm) on the basis of its multiplet pattern, since it constitutes the AB part of an ABX spin system. Long-range connectivities obtained for these signals are illustrated in structure **E**.

Considerations of the connectivity network just described, in conjunction with arguments drawn from the HMQC spectrum, permit structural segment **F** to be assembled and assigned. As a consequence, the assignments of the quaternary groups were completed. Moreover, on one hand the ^{13}C shifts of methylene carbons C-16 and C-22 were not unambiguously assigned because of the lack of resolution in the F_1 dimension (Figure 3) and, on the other hand, since we were concerned only with elucidating the bonding pattern, no stereochemical arrangement of the constituent carbons has been carried out; therefore C-29 and C-30 methyl groups assignment may be reversed.

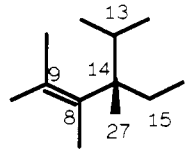
Unfortunately, since the resonances of the last two methyl protons exactly overlap, total assignment of the ^{13}C-nmr spectrum of **1** cannot be easily completed. Nevertheless, cross peaks are observed between these two overlapping methyl group signals and methylene carbons located at 34.94 and 31.04 ppm. These latter resonances were tentatively assigned to C-1 and C-12 on the basis of literature data (12).



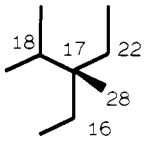
A



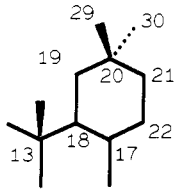
B



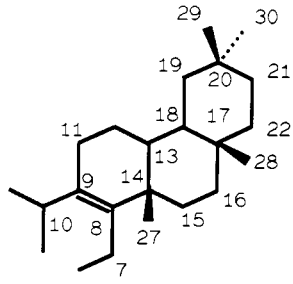
C



D



E



F

Finally, correlated peaks were observed between H-3 and H-5 and the carbons located at 24.39 (CH_2) and 20.00 (Me) ppm, respectively. These resonances were therefore ascribed to C-2 and C-25, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All spectra were recorded on a Bruker AMX-400 spectrometer in $CDCl_3$ solutions. Chemical shifts were measured in ppm relative to TMS. Resonance multiplicities for ^{13}C were established via the acquisition of DEPT spectra obtained for a proton pulse $P = \pi/2$ (CH only) and $P = 3\pi/4$ (CH and CH_3 differentiated from CH_2). The HMQC spectrum was obtained using a pulse sequence (INVBTP in the operating Bruker software) which includes the bilinear rotational decoupling (BIRD) (13) pulse to invert the magnetization of the proton not coupled to ^{13}C. This spectrum was collected with 2K × 512 data points, a data acquisition of 8 scans × 512 increments in the t_1. Spectral widths of 2050 and ± 4530 Hz were employed in the F_2 (^1H) and F_1 (^{13}C) domains, respectively. Data were processed using square sine bell functions for weighting in both dimensions; this provided a digital resolution of 2 Hz in F_2 and 17.7 Hz in F_1. The delay Δ_1 was set to 3.4 msec, while Δ_2 was empirically optimized as 400 msec. The HMBC spectrum was obtained using the standard pulse sequence (INV4LPLRND in the operating Bruker software). The spectral widths were F_2 2050 Hz and F_1 ± 10 000 Hz, allowing a digital resolution of 2 Hz in F_2 and 19.7 Hz in F_1. The delays Δ_1 and Δ_2 were set to 4 msec and 90 msec, respectively.

The mass spectrum was recorded on a Varian MAT 311 (70 eV, direct inlet, ei mode).

ISOLATION PROCEDURE.—*B. cerifera* was grown in a greenhouse in the Botanik Garden at Darmstadt. The species is cultivated continuously at Darmstadt, so living plants are available as voucher. Dry fruit is kept in the collection of the Institute of Botany. The "wax" was dissolved with $CHCl_3$. The crude material was subjected to repeated crystallization using Me_2CO/petroleum ether, yielding colorless crystals of isomultiflorenyl acetate [**1**] (mp 216–217°). The material appeared chromatographically pure.

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