LONG-RANGE PROTON–CARBON SHIFT CORRELATIONS. COMPARISON OF ONE-DIMENSIONAL WITH TWO-DIMENSIONAL TECHNIQUES

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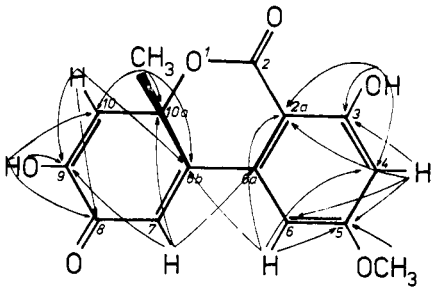
Effectiveness of two ^{13}C-detected NMR methods — two-dimensional heterocorrelated experiment and one-dimensional semiselective INEPT specified for mapping the long-range spin–spin interactions $^{n}J(C, H)$ was investigated with dehydroaltenusin (*I*), a secondary metabolite of *Alternaria tenuis* and *Talaromyces flavus* moulds.

Low molecular naturally occuring compounds often embody highly substituted aromatic systems rich in quaternary carbons. The ^{1}H NMR spectra of these compounds revealed in addition to signals of substituents (singlets of CH_3, OCH_3, OH, NH, COOH groups) predominantly signals of CH protons with only few H–H interactions. Information afforded by such spectra are frequently ambiguous and consequently, further experiments are required to elucidate the structure. A direct determination of C–C by pulse sequentions of INADEQUATE[1,2] type is frequently impossible due to a little amount of compound at hand and long relaxation times of quaternary carbons. Therefore, application of H–C long-range coupling constants ($^{n}J(C, H)$) often represents a workable possibility[3–5]. The ^{13}C-detected experiments are restricted, due to the sensitivity, to map the $^{n}J(C, H)$ coupling constants without considering the magnitude.



SCHEME 1
Structural formula of dehydroaltenusin (*I*) showing long-distance hydrogen–carbon interactions

This study concerns the effectiveness of 2D-heterocorrelated experiment[6] on comparison with the 1D-semiselective INEPT technique[7] of two ^{13}C-detected methods intended to map the $^{n}J(C, H)$ coupling constants as investigated with dehydroaltenusin (*I*) (Scheme 1), a secondary metabolite of *Alternaria tenuis*[8,9] and *Talaromyces flavus*[10,11] moulds.

RESULTS AND DISCUSSION

The Freeman–Morris sequence[6] (Fig. 1*a*) with prolonged evolution periods is the basis for two-dimensional heterocorrelated experiments aiming to map the $^{n}J(C, H)$ coupling constants; an alternative process is the pulse sequence COLOC[12,13]. Several modifications of these basic experiments were published (cf. the review article by Martin and Zektzer[14]); they (i) eliminate modulation of correlation peaks due to one-bond H–C ($^{1}J(C, H)$) coupling constants; (ii) eliminate direct correlation peaks originating from $^{1}J(C, H)$; (iii) allow various decoupling degree of proton–proton interactions in the F_1 domain. Common disadvantage of these 2D-methods is the possibility of developing a spin system also due to passive $^{n}J(C, H)$ during the refocusing period and consequently often leading to lowering or possibly disappearance of intensity of correlation peaks. Evolution of spins after neglecting the relaxation influence during the refocusing period is given by the relation

$$I_R = \sin(\pi J_a \Delta_2) \prod_i \cos(\pi J_{P(i)} \Delta_2), \qquad (I)$$

where J_a is the active heteronuclear constant through which evolution proceeds

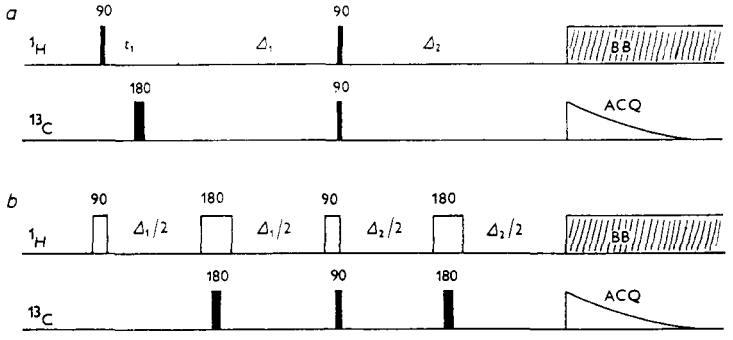


FIG. 1

NMR experiments with detection of ^{13}C spins intended for correlation through $^{n}J(C, H)$. *a* Freeman–Morris pulse sequence[6], *b* pulse sequence of the semiselective INEPT[7]

and $J_{P(i)}$ are all further passive constants of the carbon involved. Moreover, the intensity of correlation peaks is influenced by H–H coupling constants during magnetization transfer in experiments with a constant evolution period.

$$I_P = \sin(\pi J_a \Delta_1) \prod_i \cos(\pi J(H, H_i) \Delta_1) . \quad (2)$$

The adjustment of evolution periods in these experiments is, as it follows from the above relation, a matter of compromise. Influence of passive coupling constants can be eliminated during the refocusing period in special cases only[15]. A general solution of this problem does not exist within the non-selective 2D-experiment. However, the solution is encompassed in the 1D-semiselective INEPT[7] technique (Fig. 1*b*). The semiselective ^{1}H-pulses (all or only 180° in principle) employed in this experiment cause refocusation of H–H interaction in the polarization transfer and ensure the development through the active coupling constant J_a during the refocusation period only. The signal intensity in a 1D-INEPT experiment is given by expression

$$I = I_P . I_R = \sin(\pi J_a \Delta_1) \sin(\pi J_a \Delta_2) \quad (3)$$

provided a neglected relaxation, which is equal or even shorter in 1D-experiment than in two-dimensional ones. The evolution intervals of these experiments can effectively be optimized as follows from relation (*3*). This situation resembles that of the non-selective refocused INEPT[16] optimized to ^1J(C, H). The signal intensity in spectra of the semiselective INEPT is lower due to a greater relative scattering of ^nJ(C, H) in comparison with ^1J(C, H), a more noticeable relaxation during the extended evolution intervals and also to an imperfect frequency characteristic of semiselective pulses (unequal excitation of the whole ^{13}C satellite). Sensitivity of this experiment can generally be compared with that of the basic ^{13}C spectrum in the case of magnetization transfer from the CH group. Magnetization transfer from a CH_3 group is even more effective. Prerequisite for application of this experiment is a sufficient signal separation serving as a magnetization source in the ^{1}H NMR spectrum of the compound under investigation. The sufficient signal separation in the experiments with the dehydroaltenusin using perpendicular semiselective pulses was shown to be approximately 10 Hz.

Dehydroaltenusin (*I*) contains nine quaternary carbons, four CH groups and one CH_3 and OCH_3 each. Its proton spectrum is shown in the upper part of Fig. 4. The only distinguished coupling constant in the spectrum is J(H-4, H-6) = 2·4 Hz. Selection of parameters for the 2D-heterocorrelated experiment specified for correlation through ^nJ(C, H) was complicated in this case by a great range of proton resonances (ca 9·5 ppm) and necessity to discern the little separated signals in the δ 6·8 region. A convenient folding of CH_3, OCH_3 and OH signals in the F_1 domain resulted in reduction of spectral width in the hydrogen domain to δ 3·5, thereby lowering three times the number of increments in t_1 at a required digitalization. The

magnetization transfer and the refocusing period were optimized for $^{n}J(C, H) =$ $= 8$ Hz and 10 Hz, respectively. The $^{1}J(C, H)$ values for CH carbons varied in a narrow interval 162·8–166·9 Hz and therefore, no BIRD pulse[17] was applied to suppress them, but the Δ_2 interval was adjusted to $\cos(\pi^{1}J(C, H)\,\Delta_2) = 1$. In spite of this arrangement the final spectrum (Fig. 2) contained correlation peaks of the directly linked spins. Traces for individual ^{1}H resonances of this spectrum are shown in Fig. 3. Although traces of similar experiments are predominantly presented through ^{13}C signals the opposite procedure was chosen to facilitate a direct comparison of results obtained with a series of one-dimensional experiments of semiselective INEPT (Figs 4 and 5). Transfer of magnetization and refocusation for CH protons and CH_3 groups were optimized in the semiselective INEPT to $^{n}J(C, H) = 8$ and 4 Hz, respectively. Length of the semiselective pulse τ_{90} was

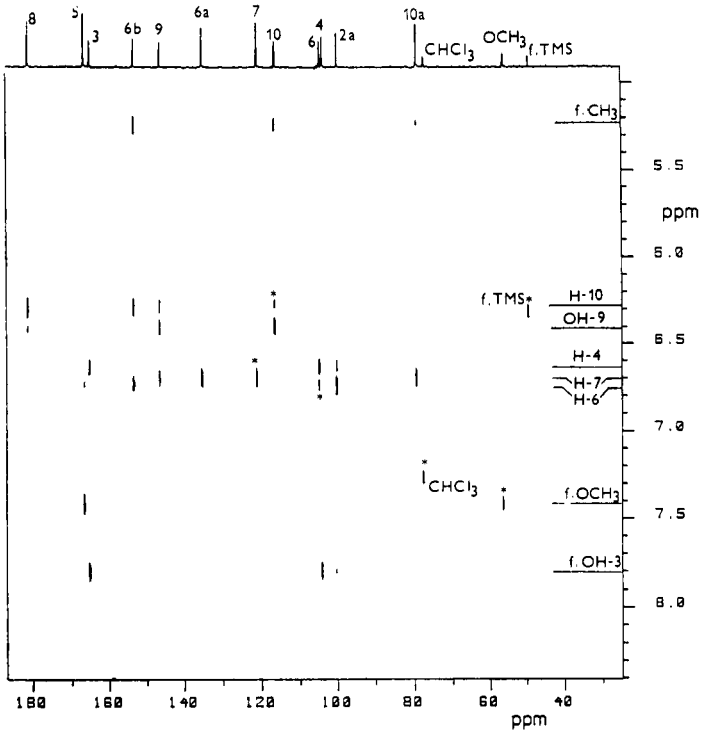


FIG. 2

2D-Heterocorrelated spectrum of dehydroaltenusin (*I*) measured by the Freeman–Morris pulse sequence optimized to $^{n}J(C, H)$. Direct correlation peaks are asterisked and the ^{1}H resonances folded in F_1 are denoted f

10 ms. Regarding the minimal separation of H-4, H-6 and H-7 protons in the ^1H spectrum of compound *I*, the 90° pulse for transferring magnetization from these protons was prolonged to 22·5 ms. A series of experiments was recorded at this length during which the offset of hydrogen pulses was gradually altered. The offset position is marked in the hydrogen part of the spectrum by arrows (Fig. 5). Virtually pure subspectra of carbons interacting with the protons H-6, H-7 and H-4 were obtained in this way (Fig. 5*a*, *c*, e).

Some differences were seen when comparing the corresponding spectra obtained by semiselective INEPT (Figs 4 and 5) with traces through 2D-heterocorrelated spectrum (Fig. 3). Different is e.g. the intensity of carbon signals in traces through CH_3 proton signals (C-10, C-10a), H-4 proton (C-5, C-2a), H-6 proton (C-4), and

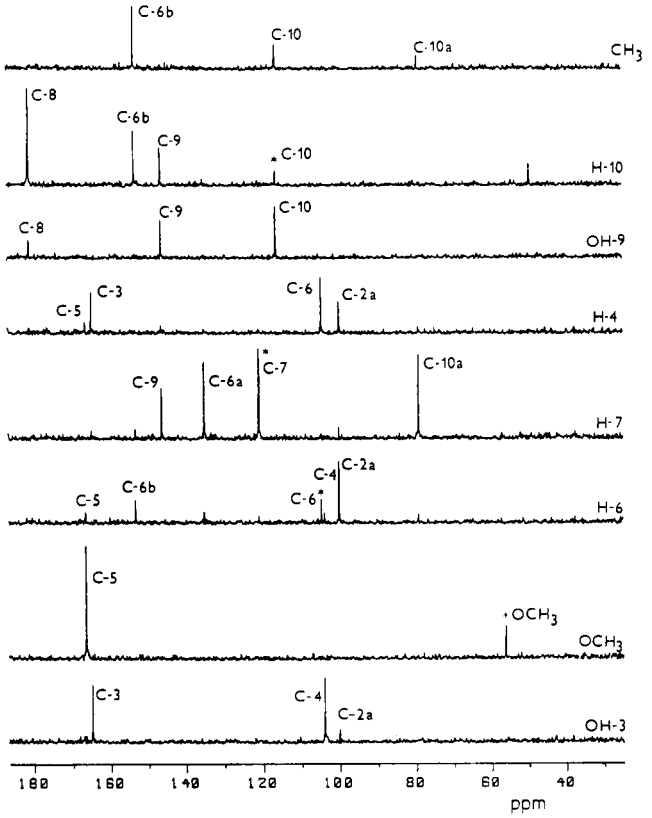


FIG. 3

Traces through hydrogen resonances of the 2D-spectrum shown in Fig. 1. Direct correlation peaks are asterisked

OH-3 proton (C-2a). These differences can be rationalized by means of relations (*1*) to (*3*) providing the couplig constants of the competent carbons are known. These were for compound *I* found experimentally by one-dimensional[18] and two-dimensional semiselective INEPT experiments[19] (Table I).

As seen, carbons in all these cases were split by several long-range coupling constants. Passive interactions lower the final intensity of correlation peaks in line with the expression (*1*). This effect is significant due to relatively great ^{n}J(C, H) values and the length of refocusing period chosen ($\Delta_2 = 1/2 \cdot 10 = 50$ ms). On the other hand, shortage of the Δ_2 interval would lead to decrease of the influence of passive ^{n}J(C, H) but would also result in an over-all lowering of all correlation peak intensities. This decrease would have a critical impact on the small active coupling constants.

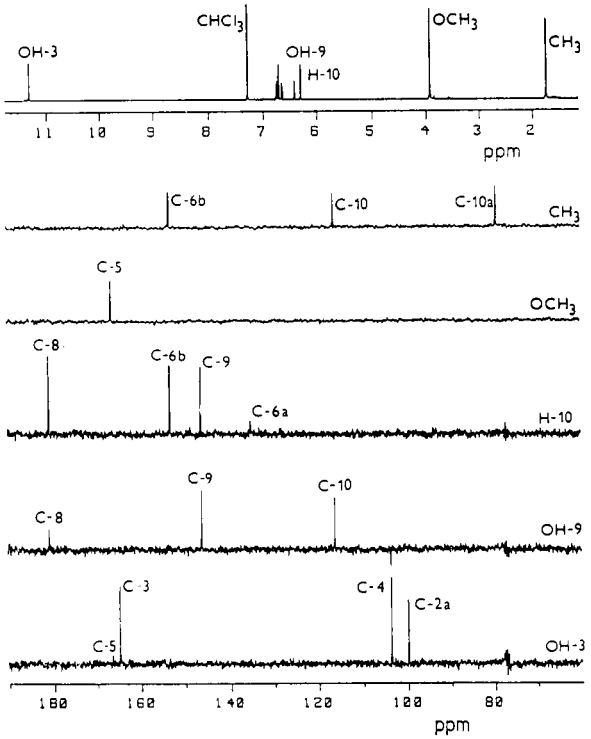


FIG. 4

Spectra of the semiselective INEPT experiment of compound *I*. The proton used for magnetization transfer is shown in the right side. The ^{1}H NMR spectrum of compound *I* is presented in the upper side

Theoretical and experimental analyses evidence that the intensity of correlation peaks in the 2D-heterocorrelated experiment is not directly proportional to the magnitude of the active coupling constant. This information was, however, preserved to some extent in one-dimensional semiselective INEPT spectra. Considering the evolution intervals to be optimized in this experiment to a sufficiently great coupling constant greater than ^{n}J(C, H) in the molecule under examination, the signal intensities of individual spectra were proportional (Eq. (*2*)) to the magnitude of the active coupling J_a (cf. e.g. ^{n}J(C, H) of H-6 proton (Table I) and spectrum in Fig. 5*a*)

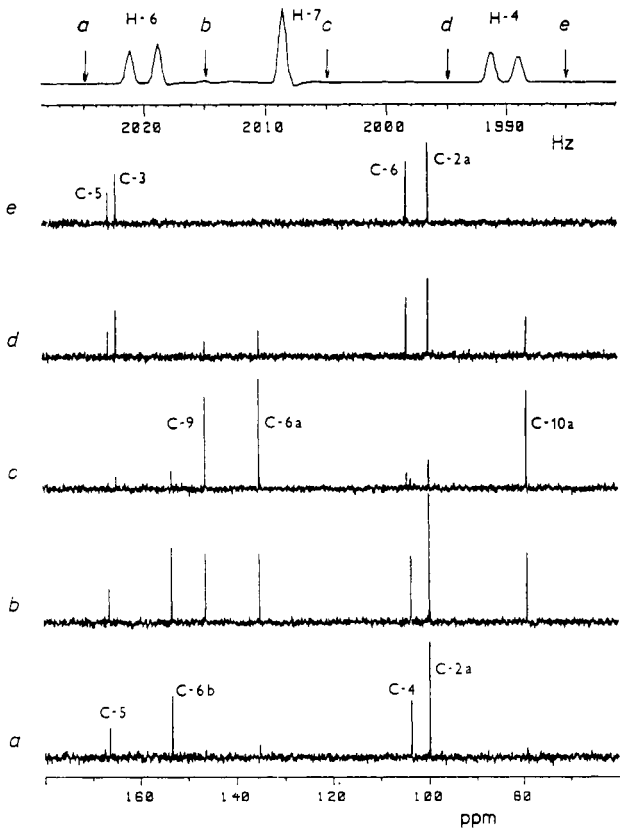


FIG. 5

Spectra of the semiselective INEPT experiment of compound *I* with magnetization transfer from protons H-4, H-6 and H-7. Spectra *a*, *b*, *c*, *d*, e were accumulated with altered offset as has been denoted by arrows in the ^{1}H NMR spectrum. Pure subspectra of carbons interacting with the protons: *a* H-6, *c* H-7, *e* H-4

So far, we presume that the differences in the spin–spin relaxation times of carbons observed can be neglected, this being substantiated in the majority of experiments. But, provided also the spin–spin relaxation times of protons are approximately equal, than the individual spectra of the semiselective INEPT can be compared as well. Complications can emerge if some ^{1}H multiplets are too broad due to splitting by many proton–proton interactions. The decrease of signal intensities of coupled carbons can be associated with an incomplete excitation due to too high pulse selectivity but not to a lower ^nJ(C, H) value.

CONCLUSION

The presented results indicate the semiselective INEPT to have several advantages against the 2D-heterocorrelated experiments optimized to ^nJ(C, H) provided the separation of signals in the ^{1}H NMR spectra is sufficient. These advantages are summarized as follows: (i) relatively higher sensitivity due to the possibility to optimize more efficiently the evolution intervals; (ii) lower time demands at a restricted number of protons for which mapping ^nJ(C, H) is necessary; (iii) greater probability to detect smaller coupling constants (3 Hz); (iv) possibility to get information on the relative magnitude of ^nJ(C, H); (v) possibility to stop accumulation at a sufficient signal to noise ratio.

The ^{1}H-detected HMBC experiment[20,21] is an attractive alternative of ^{13}C-detected experiments for determination of ^nJ(C, H) on instruments enabling inverse experiments. Recently published comparison of this experiment[22] with a semiselective INEPT showed the sensitivity of ^{1}H-detected experiment to be higher; nevertheless, problems might be encountered at a low resolution of ^{13}C resonances.

TABLE I

Hydrogen–carbon coupling constants (Hz) of selected carbons of dehydroaltenusin (*I*)

	Hydrogen							
Carbon	OH-3	H-4	H-6	H-7	OH-9	H-10	CH_3	OCH_3
C-2a	4·1	5·4	7·1	—	—	—	—	—
C-4	8·1	162·8	4·4	—	—	—	—	—
C-5	1·9	3·1	3·1	—	—	—	—	4·4
C-6	—	5·2	164·4	—	—	—	—	—
C-6b	—	—	4·4	—	—	5·9	3·1	—
C-7	—	—	—	166·9	—	—	—	—
C-10	—	—	—	—	7·8	166·4	3·7	—
C-10a	—	—	—	9·3	—	—	3·7	—

Moreover, intensity of correlation peaks can unfavourably be influenced by evolution of proton–proton interactions in the preparation period of the HMBC method. The one-dimensional INEPT experiment is less time-consuming than the affore-mentioned 2D-inversion experiment but this requires a sufficient amount of substance.

EXPERIMENTAL

The NMR spectra of compound *I* (80 mg) in deuterochloroform (2·5 ml) containing tetramethylsilane as an internal reference were run with a Bruker AM 300 instrument operating at 75 MHz for ^{13}C. The ^{1}H and ^{13}C NMR data were reported in ref.[11].

The 2D-heterocorrelated spectrum of compound *I* was measured under following conditions: relaxation time 4 s, acquisition time 0·17 s, $\Delta_1 = 62{\cdot}5$ ms, $\Delta_2 = 49$ ms; 128 spectra were accumulated in each of 64 increments. The spectra were zero filled prior to the second Fourier transformation t_1 to 256, i.e. final digitalization 4 Hz/point at a spectral width 1 050 Hz in F_1 domain. The sinus weight function in $F_2(\pi/3)$ and Gauss function ($LB = -10$ Hz, $GB = 0{\cdot}4$) in F_1 were employed for convolution. The total experiment period was 9·5 h.

The evolution intervals in the semiselective INEPT experiment were optimized according to dependence $\Delta_1 + 2\tau_{90} = \Delta_2 + \tau_{90} = 1/2{}^{n}J(C, H)$ for CH ($^{n}J(C, H) = 8$ Hz), or $1/5{}^{n}J(C, H)$ for CH_3 protons ($^{n}J(C, H) = 4$ Hz), where τ_{90} stands for the length of a 90° semiselective pulse. A semiselective pulse $\tau_{90} = 22{\cdot}5$ ms was employed for the experiment illustrated in Fig. 5 and $\tau_{90} = 10$ ms pulse for all other experiments. The relaxation interval for CH protons at 1 000 accumulations was 4 s for each spectrum, measuring time 75 min; for CH_3 2 s, 400 accumulations, 0·5 s acquisition time and 0·28 h measuring time. The spectrum was twice zero-filled and multiplied by the experimental function ($LB = 1$ Hz). The total measuring time was 4·31 h.

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