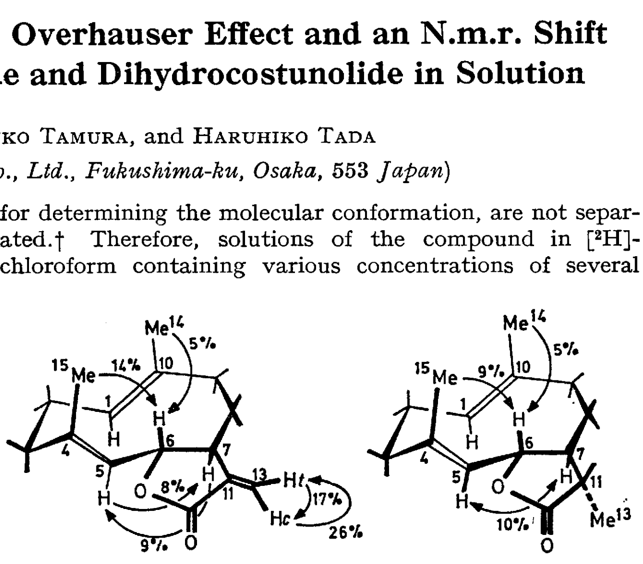
Simultaneous Application of the Nuclear Overhauser Effect and an N.m.r. Shift Reagent. Conformations of Costunolide and Dihydrocostunolide in Solution

By KAZUO TORI,* ISAO HORIBE, YOUKO TAMURA, and HARUHIKO TADA
(*Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka,* 553 *Japan*)

Summary Conformations of the ten-membered ring sesquiterpene lactones costunolide (**1**) and dihydrocostunolide (**2**) in [^{2}H]chloroform have been determined by intramolecular inter-nuclear Overhauser effect measurements in the presence of an n.m.r. lanthanide shift reagent, [$^2H_{27}$]Eu(fod)$_3$; care must be taken in applying these techniques simultaneously.

IT HAS been suggested[1] that simultaneous application of the intramolecular inter-nuclear Overhauser effect (NOE)[2] and a lanthanide shift reagent[3] in ^{1}H n.m.r. spectroscopy should provide more useful information about the structure and stereochemistry of organic molecules than is obtainable by the former method alone. However, neither a simple example of the simultaneous use of these techniques nor an experiment on the relationship between NOE enhancements and the concentration of a paramagnetic shift-reagent has so far been reported. We report here the determination of conformations of two ten-membered ring sesquiterpene lactones isolated in this laboratory,[4] costunolide (**1**) and dihydrocostunolide (**2**), in [^{2}H]chloroform, by the application of NOE studies in the presence of tris-(1,1,1,2,2,3,3-heptafluoro-7,7-[$^2H_6$]dimethyl[8-$^2H_3$]octane-4,6-dionato)-europium(III), [$^2H_{27}$]Eu(fod)$_3$, together with some suggestions and comments on the simultaneous applications of these techniques to structural problems.

The 100 MHz ^{1}H n.m.r. spectra of (**1**) in several solvents show that signals due to 1-H, 5-H, 6-H, and 7-H, important for determining the molecular conformation, are not separated.† Therefore, solutions of the compound in [^{2}H]-chloroform containing various concentrations of several kinds of shift reagents were examined; [$^2H_{27}$]Eu(fod)$_3$ was chosen as the best shift reagent in the present case since it has a stronger shift power and gives less line-broadening. The chemical shifts in the absence of the reagent and the *S*-values (downfield shifts; in parentheses)‡ obtained for 1-, 5-, 6-, 7-, 13*t*-, 13*c*-, 14-, and 15-H are as follows: δ 4·83 (0·93), 4·78 (2·10), 4·53 (3·87), 2·50 (3·55), 5·51 (3·06), 6·24 (7·01), 1·43 (0·80), and 1·69 (0·86), respectively.



(1) (2)

FIGURE. *Molecular conformations and NOE values† for costunolide* (**1**), *determined in the presence of a* 0·40 *molar ratio of* [$^2H_{27}$]Eu(fod)$_3$, *and dihydrocostunolide* (**2**), *determined in the presence of a* 0·65 *molar ratio of the shift reagent.*

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† The spectra were taken with a Varian HA-100 spectrometer in the frequency-swept and internal Me$_4$Si-locked mode at 30 °C. Sample concentrations were 0·27 mol l^{-1} in [^{2}H]chloroform. The NOE experiments were performed as described previously,[10] and NOE values were obtained as percentage increases in integrated signal intensities (accuracies are within $\pm 2\%$). NOE [*i*-H]→*j*-H, represents an NOE on the *j*-H signal when the *i*-H signal was completely saturated by double irradiation.[2,10]

‡ Slopes of initial, linear parts of the shift curves obtained were used as shift values (*S*-values). The *S*{[$^2H_{27}$]Eu(fod)$_3$}/*S*{[$^2H_{27}$]Pr-(fod)$_3$} ratios were fairly constant for (**1**) and (**2**). This fact may be taken as indicating that the contact-term contribution is very small, provided that the structures of the complexes between the lactones and both reagents are the same.[8]

NOE measurements were then performed on degassed solutions of (**1**) containing various concentrations of $[^{2}H_{27}]$-Eu(fod)$_3$. The NOE values† for all observable pairs of signals were plotted against molar ratios of shift reagent to substrate. The NOE [13*t*-H] → 13*c*-H† decreased rapidly with the initial increase in shift-reagent concentration (45, 34, 33, 26, 23, 17, 18, and 17% at 0, 0·03, 0·06, 0·10, 0·20, 0·40, 0·60, and 0·80 molar ratios, respectively), while the reverse NOE [13*c*-H] → 13*t*-H decreased to a lesser extent (30, 28, 28, 27, 27, 26, 26, and 25% at the same molar ratios, respectively). The other NOEs observed were also slightly reduced by the reagent.

This trend of decrease in NOE by the paramagnetic shift reagent closely resembles that reported for the reduction of ^{13}C-^{1}H Overhauser enhancements in proton-decoupled ^{13}C n.m.r. spectra in the presence of a paramagnetic species.[5] A theoretical basis for the latter observation, outlined by Gutowsky and Natusch,[6] is applicable to ^{1}H-^{1}H Overhauser effects, as in the present case. Reduction in NOE enhancements will depend directly on a very sensitive function[7] (r^{-6}) of the distance, r, between the electron spin, *i.e.* that of Eu^{3+}, at the binding site, and the proton under observation, as long as the contact-term contribution[8] is negligibly small, the scalar proton-electron coupling term being omitted.‡ The preferential reduction of the NOE of 13*c*-H observed here thus implies that 13*c*-H is the closest to the Eu^{3+} bound to the –O·CO– grouping.

Fortunately, the decrease in the NOE was fairly low even at considerably higher concentrations of the shift reagent, probably owing to the low proton–electron relaxation efficiency of the reagent.[6] The NOE technique may thus be widely applicable in the presence of shift reagents. Caution should be exercised, however, when a proton to be observed is very near to the binding site(s); this can generally be determined by use of *S*-values.[3,8]

Our results show that out of four possible conformations indicated by a molecular model examination, that of the ten-membered ring of (**1**) was as illustrated. In the spectrum of a solution of (**1**) containing a 0·40 molar ratio of $[^{2}H_{27}]$Eu(fod)$_3$, important signals were found to be completely separated. The conformation is very similar to that determined by *X*-ray analysis of a crystal of a 1 : 2 adduct of (**1**) with silver nitrate.[9]

Similarly, NOE measurements on a solution of (**2**) in $[^{2}H]$chloroform containing a 0·65 molar ratio of $[^{2}H_{27}]$Eu-(fod)$_3$ demonstrated that the ten-membered ring adopts the conformation illustrated. The chemical shifts in the absence of the reagent and the *S*-values (downfield shifts; in parentheses)‡ obtained for 1-, 5-, 6-, 7-, 11-, 13-, 14-, and 15-H are as follows: δ 4·80 (1·25), 4·63 (2·57), 4·58 (4·34), obscured (4·40), obscured (9·75), 1·25 (6·20), 1·43 (0·85), and 1·69 (0·85), respectively. Furthermore, the conformation of a new germacranolide, parviolide, isolated from *Magnolia sieboldii* K. Koch and identified as 15-acetoxycostunolide,[4] has been shown by the present technique to be very similar to that of (**1**).

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