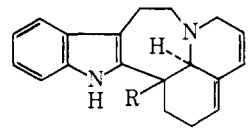
The Fourier Transform Difference Spectra Method. An Application to Structural Elucidation of Andranginine, a Novel Indole Alkaloid

Sir:

The NMR difference spectrum technique has been applied mostly to large molecules to identify the spectral position of methyl lines[1] or to simple spin systems to prove the possibility of realizing INDOR experiments in the FT mode.[2] In this communication we report a different application of this technique which has enabled us to identify all the protons of a new alkaloid, andranginine (**1**) and therefrom to propose a structure for it.



1, R = CO_2CH_3
2, R = CH_2OH

In ordinary double resonance operation, two spectra, one normal and the other obtained with an irradiating rf field applied to an NMR line under study, are compared and from the relative changes of spectrum configuration between the two, ^1H-^1H couplings can be deduced and finally identified. The visual inspection of the two spectra may be rendered easier, however, if only the difference of the two is recorded in lieu of the whole spectrum. This technique normally can be performed in two steps with a FT NMR spectrometer. The first step consists of accumulating in the ordinary manner a certain number of free induction decay (fid) signals. During the following step the "monitored" proton is subjected to a monochromatic rf irradiating field while the same number of fid signals are being accumulated. Subtraction is then performed between the two results and this difference is Fourier transformed. The spectrum recorded is then the difference spectrum. The NMR line of the monitored proton reappears after subtraction since it is saturated half of the experiment time, whereas the signals of the protons coupled to it, owing to the change of pattern of the irradiated spectrum, give a nonzero response. All the lines which are not related to the proton under study will disappear after subtraction except those which are close to the irradiation point and so are subjected to a decrease in intensity and to a slight frequency shift due to Bloch-Seegert effects.[3]

The seven difference spectra of Figure 1 were recorded on a laboratory built 240-MHz spectrometer using a four-phase rotation sequence by means of which a single computer memory was needed.[4] Each plot was obtained in 256 runs in order to ensure an acceptable signal-to-noise ratio. Except for plot no. 4 which shows a weaker response, all other spectra indicate at first glance the relative couplings between protons.

These spectra have been obtained with a sample of andrangininol, **2**, an alkaloid which is derived from andranginine, **1**. These represent a new type of indole alkaloid found in an Apocynaceae plant, *Craspidospermum Verticullatum Boj. var. petiolare*.[5] Mass spectral and ^{13}C NMR data did not permit its classification into any known alkaloid group, therefore a detailed study of its high field ^1H NMR spectrum was undertaken with the hope of determining the carbon skeleton.

The difference spectrum 1 shows the coupling between the two olefinic protons, H-14 and H-15, and a proton sit-

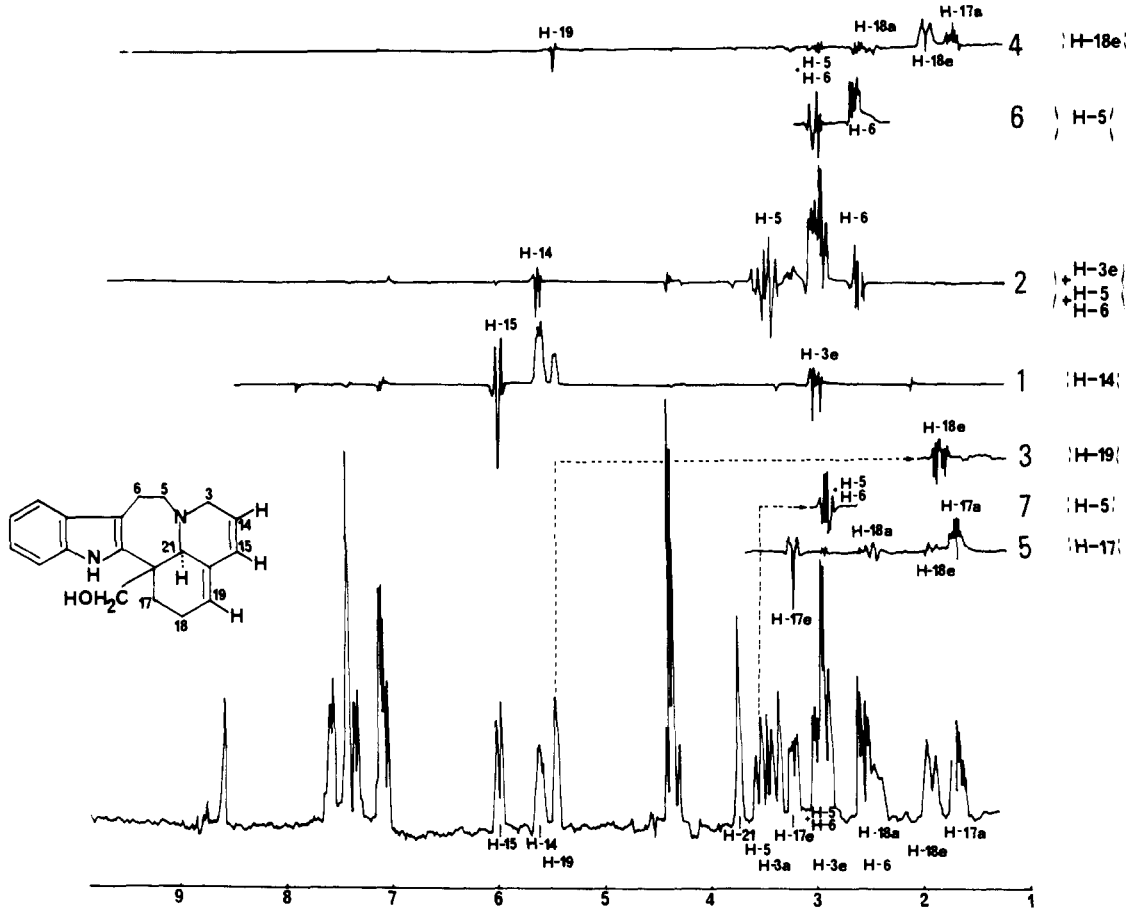


Figure 1.

uated at δ 3.0, H-3e. Although the proton H-3e is not observed, its coupling constants can be measured on the difference spectrum. From the apparent asymmetry of the H-3e doublet of doublets and from difference spectrum 2, it appears that H-3e is coupled either with the doublet at δ 3.3 or with the triplet at δ 3.5. This ambiguity is removed by monitoring the triplet giving a response within the assembly of peaks around δ 3.0, but at a frequency lower than that of H-3e (plot 7). H-3e is thus coupled with the doublet at δ 3.3 due to H-3a which is linked to the same carbon as shown by their large coupling constant (J = 16 Hz).

The last olefinic proton is coupled with a small coupling constant to the doublet at δ 1.9, which in turn gives difference responses at δ 1.6 and 2.5 (H-17a and H-18a). Finally, H-17e is coupled with H-18e, H-18a, and H-17a (plot 5). The results, in addition to the measure of coupling constants enable us to place the five protons on a unit such as $=CHCH_2CH_2$.

The four remaining protons, δ 3.5, 2.9 (two protons), and 2.6 can be attributed to the tryptamine moiety certainly present here. Coupling between them can be seen in plots 2, 6, and 7. Definitive assignments of protons on tryptamine carbons 5 and 6 could only be made after displacement of the triplet at δ 3.5 to higher frequencies by protonation of the nitrogen with trifluoroacetic acid.[6] As this proton is not coupled with the doublet of doublets at δ 2.6, it is one of the H-5 protons and the other one is one of the H-6.

Using the above-mentioned elements to reconstitute the "puzzle", we propose the pentacyclic structure **2** for andranginine. The X-ray crystal structure[7] has confirmed this hypothesis and has established the stereochemistry of the C, D, and E ring junctures as trans–trans.

Acknowledgment. The authors wish to express their thanks to Drs. C. Kan-Fan, H.-P. Husson, and P. Potier for the sample of andranginine and for stimulating discussions along this work.

References and Notes

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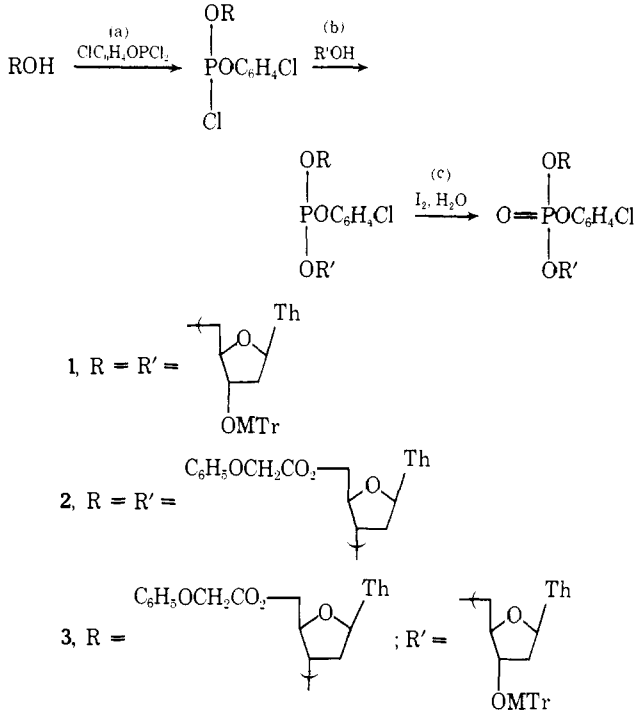
Received September 21, 1974

Phosphite Coupling Procedure for Generating Internucleotide Links[1]

Sir:

The phosphotriester procedure utilizing activation of a nucleoside phosphodiester and condensation of the active phosphoryl compound with a nucleoside has proven useful in synthesizing short oligonucleotide chains in quantity.[2] For routine synthesis of long polynucleotides, however, more efficient and rapid coupling methods are needed. Attempts to prepare phosphotriester derivatives of oligonucleotides by stepwise condensation of nucleosides with alkyl or aryl phosphorodichloridates [$(RO)P(O)Cl_2$] have been only partially successful;[2f-h] the reactions are slow and the yields are low.

We report in this communication a new procedure for generating nucleotide phosphotriesters that shows considerable promise in polynucleotide synthesis. The sequence is based on the remarkable reactivity of phosphorochloridites [$ROPCl_2$ and $ROP(OR')Cl$] toward alcohols in tetrahydrofuran at low temperatures and on the facile oxidation of phosphites to phosphates by iodine and water.[3]



A typical example is provided by the synthesis of the *o*-chlorophenyl ester of bis(mono-*p*-methoxytrityl)thymidylyl[5′-5′]thymidine (**1**). 3′-*O*-Mono-*p*-methoxytritylthymidine[4] was stirred at −78° with 0.55 equiv of *o*-chlorophenyl phosphorodichloridite[5] and 1.1 equiv of pyridine in tetrahydrofuran for 30 min. The mixture was warmed to ca. −10° in <1 min and treated with an equivalent of iodine in tetrahydrofuran–water (2:1). Oxidation proceeded about as rapidly as the iodine was added, as indicated by loss of the iodine color. Isolation of the solid product and purification by chromatography on silica plates afforded **1** (76%); mp[6] 140–143°; R_f (EtOAc–THF, 2:1) 0.38; λ_{max} (MeOH) 265 nm (ε 20,100); λ_{min} 251 nm (ε 17,200). Anal. Calcd for $C_{66}H_{62}ClN_4O_{14}P$: C, 65.97; H, 5.20; N, 4.66. Found: C, 65.82; H, 5.19; N, 4.57.

Compound **2**, a 3′-3′ dinucleoside phosphate derivative, was similarly prepared from 5′-*O*-phenoxyacetylthymidine;[7] yield of isolated **2**: 66%; mp[6] 91–94°; (R_f EtOAc–THF, 2:1) 0.31; λ_{max} (MeOH) 265 nm (ε 21,500); λ_{min} 234 nm (ε 5030).

For synthesis of a triester with a natural 3′-5′ internucleotide link (compound **3**), 5′-*O*-phenoxyacetylthymidine was treated (10 min) with *o*-chlorophenyl phosphorodichloridite (0.9 mol equiv) and 2,6-lutidene (3.6 equiv) in tetrahydrofuran at −78°. 3′-*O*-Mono-*p*-methoxytritylthymidine (0.5 equiv) was added; then the mixture was stirred