further work is in progress aimed at the determination of the positions of attachment.

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**NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY: ABNORMAL SPLITTING OF ETHYL GROUPS DUE TO MOLECULAR ASYMMETRY, III**

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The protons of a methylene group removed by one or more bonds from a center of molecular asymmetry may be magnetically nonequivalent and display AB-type nuclear magnetic resonance (n.m.r.) spectra.1–4 It has been suggested that the chemical shift between the two methylene protons arises from unequal populations of the possible rotational conformations.2 However, even assuming equal populations and rapid interconversion of the three conformers I, II, and III, N and H are always distinct and identifiable, since no two conformers are identical except for interchange of N and H.5,6 This “intrinsic asymmetry” of the methylene group might also be the cause of the observed magnetic nonequivalence of the methylene protons.

The n.m.r. spectrum of the —O—CH₂— protons of cyclopropylmethylicarbinyl ethyl ether IV (Fig. 1) is of the AB type (split by the methyl protons) and results from proximity of a center of molecular asymmetry to the methylene protons. To investigate the possibility that the difference in chemical shift between these methyl-
ene protons arises from an intrinsic asymmetry depending only on the symmetry characteristics of the molecule, we have examined the n.m.r. spectrum of cyclopropylmethylcarbinyl methyl-\textit{d}_1 ether (V).\textsuperscript{7} Substitution of a deuteromethyl group for the ethyl group of IV would be expected to eliminate any conformational

![Diagram of molecular structures](image)

**Fig. 1.**—Nuclear magnetic resonance spectra of the methylene protons of cyclopropylmethylcarbinyl methyl-\textit{d}_1 ether and cyclopropylmethylcarbinyl ethyl ether at 60 Mc/sec.

![Diagram of molecular structures](image)

**Fig. 2.**—Methine and methylene proton resonances of 2-chloro-4-ethyl-3-phenylcyclobut-2-\textit{ene} in carbon tetrachloride at 60 Mc/sec with tetramethylsilane (0 cps) as external standard. The values of coupling constants to the methine proton at the 4-position are $|J_{AX}| = 6.9$ cps and $|J_{BX}| = 4.3$ cps.

preference of the methylene protons with respect to the asymmetric center; hence, observation of an AB-type methylene spectrum for V would be evidence for an observable chemical shift arising from intrinsic asymmetry in the molecule.

After account is taken of coupling between the protons and deuterium, which has a nuclear spin of 1, the methylene resonance of V is clearly $A_2$ (Fig. 1). The magnetic equivalence of these methylene protons of V appears to us as strongly sug-
gestive that conformational preference is the factor responsible for the magnetic nonequivalence of the methylene protons of IV.

Further support for this conclusion is provided by the n.m.r. spectrum of the substituted cyclobutenone VI (Fig. 2). The resonance centered on 113 cps is of the methylene protons of the ethyl group of this compound and is the rather complicated AB part of an ABCX system. The resonance of the methine proton at the 4-position of the cyclobutene ring is centered on 233 cps and is split into two equally intense doublets, rather than a 1:2:1 triplet. This splitting is most simply explained as the result of unequal coupling between the methine proton and the two adjacent methylene protons, arising from a preference for a conformation for the molecule in which the methine proton is trans to one methylene proton and gauche to the other.

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† Contribution No. 2842.

3 Shafer, F. R., D. R. Davis, M. Vogel, K. Nagarajan, and J. D. Roberts, these PROCEEDINGS, 47, 49 (1961).
7 Prepared by reaction of methyl bromide-d3 with sodium 1-cyclopropylethoxide.

A CHANGE FROM NONSENSE TO SENSE IN THE GENETIC CODE

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For the genetic information in a cistron to be translated into a polypeptide chain, each coding unit in the nucleotide sequence must correspond to one of the twenty or so amino acids. If not every possible sequence corresponds to an amino acid, mutations that substitute one base for another could, in certain cases, cause the continuity of the information to be interrupted, and such “nonsense” mutations would block completion of the polypeptide chain.

By virtue of a mutant with special properties, it is possible to identify nonsense mutations within the A cistron of the rII region of phage T4. In this paper the criterion for nonsense is applied to certain “ambivalent” rII mutants,1 i.e., ones whose phenotypes can be reversed by suppressor mutations in the bacterial host, Escherichia coli. The results show that an ambivalent mutation that behaves like nonsense in one bacterial host may nevertheless make sense in a second (suppressor containing) host. This suggests that a suppressor mutation in the bacterium can result in addition to the cell’s dictionary of a new sensible coding unit, constituting a change in the genetic code of the bacterial cell.

The rI589 System as a Genetic Test for Nonsense Mutations.—The rII genetic region of phage T4 consists of two contiguous regions, A and B, each behaving as a