plete inhibition is rather high, *viz.* 5 × 10⁻⁴
M, probably on account of the presence of
comparatively large amounts of inactive
protein in the enzyme preparation. The
inhibition was not reversed by dialysis.
However, glutathione in slightly higher
than equimolar concentrations abolished
the inhibition completely.

Chloroacetophenone in high concentrations
inhibited the enzyme, to a certain extent,
viz. 90 % at 2.5 × 10⁻³ M. *Iodoacetamide*
had a similar effect.

Garlic extract contains allicin which is
known as an inhibitor of SH-groups [7]. It
may be noted that garlic extract, prepared
in the proportion 1:200 w/v, inhibits the
enzyme completely. Glutathione counter-
acts this inhibition.

The inhibition of the histamine methyla-
tion by *p*-chloromercuribenzoate and the
counteraction of glutathione are taken as
evidence that SH-groups might be essential
for the activity of the methylating enzyme.
The inefficiency of *o*-iodosobenzoate may
be explained by a protecting effect of other
proteins present.

This work is part of investigations made
possible by grants from the *Swedish Medical
Research Council.*

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Long Range Coupling of Nuclear Spins in Some Olefinic and Acetylenic Compounds

RAGNAR A. HOFFMAN and
SALO GRONOWITZ

*Institutes of Physics and Chemistry, University
of Uppsala, Uppsala, Sweden*

In view of the growing interest in the
theory of the indirect coupling of nuclear
spins [1-4] we wish to report some recent

findings in connection with our study of
long range couplings in proton magnetic
resonance spectra.

In an earlier communication [7] one of us
suggested that the coupling of methyl
group protons over four bond distances
with protons bonded to unsaturated carbon
atoms should be understood in terms of
hyperconjugation.

The need for new evidence for the exist-
ence of hyperconjugation in the ground
state of unsaturated organic molecules has
become obvious as Dewar and Schmeising
have recently pointed out [8] that the earlier
evidence is inconclusive. It is hoped that
a continued investigation of the above
mentioned spin couplings may provide
such evidence.

In order to verify the interpretation
given in Ref.[7], we have tested two predic-
tions that follow from the discussion pre-
sented there.

1. The interaction of the π-electron, on
an unsaturated carbon atom, with the pro-
tons of an attached methyl group is ap-
proximately equal to its interaction with
a proton directly bound to the unsaturated
carbon.

2. The coupling of methyl group protons
(to other protons) over triple bonds should
be considerably larger (about twice) than
those over double bonds.

These predictions were tested in a num-
ber of substances. Thus we find, that the
coupling between the two non-equivalent
methyl groups in tiglaldehyde and in β-
bromoangelic acid methylester are 1.0 c/s
and 1.5 c/s, respectively *, or of the same
order of magnitude as J_{ab}^I in *trans*-croton-
aldehyde (I), which equals 1.6 c/s or the
analogous coupling in *trans*-propenylben-
zene, which is reported [9] to equal 1.8 c/s.

Since methyl acetylene is a gas at room
temperature we have instead studied pro-
pargyl alcohol and propargyl chloride.
Here the coupling of the methylenic pro-
tons with the acetylenic proton equals 2.4
c/s and 2.6 c/s, resp., in agreement with the
values 2.6 — 2.8 c/s for the propargyl halides
reported by Whipple *et al.*[10]

When the acetylenic hydrogens are sub-
stituted by methyl groups, the long range
coupling constants (here between the me-
thyl and methylene protons) remain vir-

* All coupling constants reported here are
given to ± 0.1 c/s. We use the symbol J_{ab}^I
to denote the coupling constant J between
groups a and b in compound I.

tually unchanged and are found to be 2.4 c/s and 2.5 c/s in the 2-butyne-1-ol and 1-chloro-2-butyne, resp., in perfect agreement with our first prediction. Prediction 2) is reasonably well fulfilled.
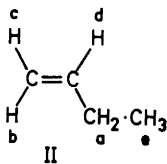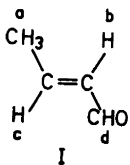
In two compounds studied by us, crotonaldehyde (I) and tiglaldehyde, relative signs of the methyl group coupling constants could be determined. In tiglaldehyde the 40 Mc/s spectrum agrees with that calculated with second order perturbation theory [11] if all coupling constants are assumed to have equal sign. An Y_3 ABX-treatment [12] of the crotonaldehyde (I) spectrum at 40 Mc/s gave two possible assignments: one assuming J_{ab}^I and J_{ac}^I having equal sign, the other assuming unlike signs of these coupling constants. A well resolved (10^3) spectrum obtained at a radio frequency of 25 Mc/s * favours the latter assignment.

This is in agreement with the results obtained for the similar compound *trans*-propenylbenzene and for but-1-ene (II) by other workers [9,13]. Alexander concluded [13] that J_{ad}^{II} in this latter compound is of opposite sign to J_{ab}^{II} and J_{ac}^{II}. He ascribes the negative value to J_{ad}^{II}. Because the methylene protons have a large shift to the vinyl protons and the influence of the coupling constant J_{ac}^{II} seems to have been excluded from the calculation, we assume that the reverse assignment of signs would give the same calculated spectrum. If this assumption is valid we prefer to ascribe the negative coupling in crotonaldehyde (I) to

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* We are greately indebted to Dr. H. Primas and Dr. P. Bommer (Zürich) for providing this spectrum.

J_{ab}^I. The cases of tiglaldehyde and crotonaldehyde would then form a neat parallel to the VB-calculations of π-electron couplings in even-alternant hydrocarbons by McConnell [5] (*cf.* also a recent note by McLachlan [14]), and also with the relative signs of coupling constants of a conjugated diene as reported by Elvidge and Jackmann [15].

A more comprehensive discussion of the above mentioned results with reference to related observations by other workers in this field will be submitted for publication at a later date.

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