1).[13] Under similar incubation conditions, 4-acetamidosalicylic acid showed only an unchanging amount of reversible inhibition with time,[13] thus indicating that the alkylating action of the iodoacetyl group of I is necessary for the irreversible inhibition by I and that the effect of I is not due to chelation or some other action by the salicylate structure.

Further evidence that binding of I to the active site of GDH is a necessary prerequisite for irreversible inhibition was obtained.

(1) The reversible GDH inhibitor, isophthalate,[14] at 16 mM. concentration, reduced the rate of inactivation by I to about one-half.[13] Protection against irreversible inhibition by a competitive inhibitor is considered to be strong evidence that the active site is involved in the irreversible inhibition.[15,17,18]

(2) The rate of inactivation should be dependent upon the dissociation constant for III + IV ↔ V, that is, the K_i. For this purpose I was compared with 4-(iodoacetamido)-benzoic acid (II).[19] First, the reactivities of the halogen in I and II were shown to be identical.[12] Second, I and II showed reversible inhibition of GDH with average $K_i = 6.9 \times 10^{-4}$ and 5.4×10^{-3}, respectively,[20] an eight-fold difference. When I or II was incubated with GDH·DPNH at 37°, I inactivated the enzyme at eight times the rate of II, in agreement with the ratio of the two K_i values.[21]

(3) It can be calculated from the K_i of I that doubling the concentration of I from 1 mM. to 2 mM. should give only a 1.23-fold increase in the inactivation rate, rather than a two-fold increase if tail-alkylation occurred. Simultaneous incubation experiments did indeed show a rate saturation effect, the observed ratio of the rates being 1.23.[13]

That I was not a general enzyme poison was shown by the observation that hexokinase·ATP was inhibited neither reversibly nor irreversibly under the conditions used for GDH; however. LDH and glucose-6-phosphate dehydrogenase were irreversibly inhibited and studies of the type described for GDH and I are progressing.

This new type of non-classical antimetabolite that most probably operates by *exo*-alkylation should have broad utility for drug design. In addition, properly designed *exo*-alkylating irreversible inhibitors could fill the demand for

(13) All incubation experiments were duplicated at least twice.

(14) W. S. Caughey, J. D. Smiley and L. Hellerman, *J. Biol. Chem.*, **224**, 591 (1957).

(15) Isophthalate has been shown[11] to be a competitive inhibitor of L-glutamate and a non-competitive inhibitor of α-ketoglutarate. Isophthalate most probably combines with the active site of GDH in both directions, but gives non competitive kinetics in the one direction due to a two-step rate phenomenon related to that recently described for acetylcholinesterase.[16]

(16) R. M. Krupka and K. J. Laidler, *J. Am. Chem. Soc.*, **83**, 1445, 1448, 1454 (1961).

(17) J. A. Thoma and D. E. Koshland, Jr., *J. Mol. Biol.*, **2**, 169 (1960).

(18) That this protection was not due to interaction of isophthalate and I was shown in an independent thiosulfate-type experiment.[12]

(19) S. Sakai, G. Saito, and A. Sato, *Chem. Abstr.*, **51**, 16940 (1957).

(20) The K_i values were calculated from K_p and K_m, obtained from a Lineweaver–Burk plot according to ref. 10, pp. 22–24.

(21) That these results were not due to traces of heavy metal ions was shown by a highly sensitive spectrographic analysis of the I, II and 4-acetamidosalicylic acid used in these experiments.
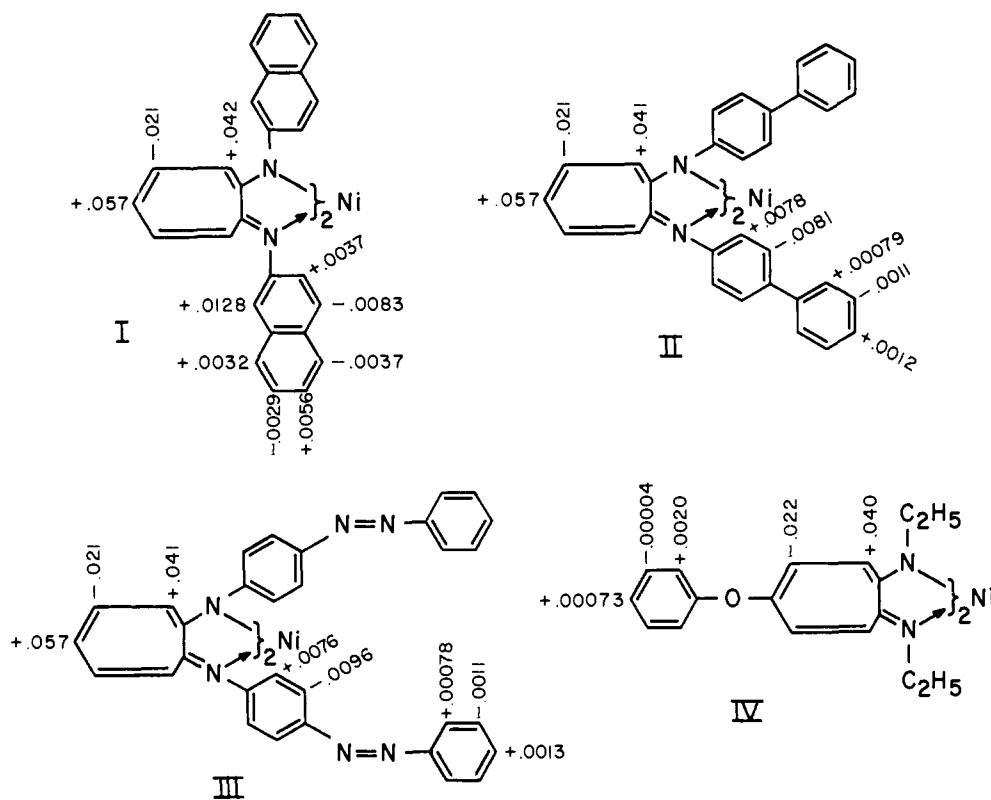
relatively specific agents needed to label the active sites of enzymes for enzyme mechanism studies.[22,23]

(22) Reference 10, pp. 486–502.
(23) Ref. 9, p. 618.
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ELECTRON SPIN DENSITY DISTRIBUTIONS IN CONJUGATED SYSTEMS BY N.M.R.

Sir:

It was found[1] that π-electron spin densities on the conjugated ligands of some paramagnetic nickel(II) chelates of aminotroponeimines could be determined from n.m.r. studies. It now appears that substituted nickel aminotroponeimineates can be employed for a general study of conjugative effects and spin density distributions in π-systems. Some preliminary results of this study are reported below. In particular, it is shown that spin densities are transmitted through N, O and S atoms connecting conjugated systems.

A positive spin density is placed on the p π orbital of nitrogen of nickel(II) aminotroponeimineates as a result of the nickel–nitrogen bonding. This spin density is distributed throughout the π-system of the ligand. Spin densities on sp^2 carbon atoms to which hydrogen atoms are bonded are manifested in the proton magnetic resonance spectrum by large high field (positive carbon spin densities) and low field (negative carbon spin densities) shifts.[2] These shifts, $\Delta H/H$,[3] are produced by isotropic hyperfine contact interactions[4] and are related to the nuclear hyperfine coupling constants, a_i, by

$$\frac{\Delta H_i}{H} = -a_i \frac{\gamma_e}{\gamma_H} \frac{g\beta S(S+1)}{6kT} \tag{1}$$

Each a_i is related to its carbon atom spin density ρ_{ci}, by[5]

$$a_i \cong Q\rho_{ci} \tag{2}$$

For aromatic C–H fragments, $Q \cong -22.5$ gauss.[6]

Analyses of the n.m.r. spectra of the nickel aminotroponeimineates[7] (obtained on 0.1–0.2 molar solutions of chelate in $CDCl_3$) with respect to assignment of resonances to unique ligand hydrogen atoms were facilitated by relative resonance intensities, nuclear spin-spin structure, and expected signs of spin densities based on simple valence bond considerations. A paramagnetic ⇌ diamagnetic equilibrium exists for these chelates in solution.[1] The spin density distributions shown in Fig. 1 refer to the paramagnetic state ($\mu_{eff} = 3.2$ BM.)

(1) W. D. Phillips and R. E. Benson, *J. Chem. Phys.*, **33**, 607 (1960).

(2) H. M. McConnell and D. B. Chesnut, *ibid.*, **28**, 107 (1958).

(3) Where $\Delta H = H_{chelate} - H_{ligand}$. Both ligand and chelate were internally referred to $(CH_3)_4Si$ to eliminate bulk susceptibility effects.

(4) E. Fermi, *Z. Physik*, **60**, 320 (1930).

(5) H. M. McConnell, *J. Chem. Phys.*, **24**, 632 (1956).

(6) S. I. Weissman, T. R. Tuttle, Jr., and E. de Boer, *J. Phys. Chem.*, **61**, 28 (1957).

(7) Prepared from the appropriate ligand and nickel chloride; see W. R. Brasen, H. E. Holmquist and R. E. Benson, *J. Am. Chem. Soc.*, **83**, 3125 (1961).

Fig. 1.—Spin density distributions on some nickel(II) aminotroponeimineates.

An example of such an analysis involving ten nucleus-electron coupling constants is provided by the 2-naphthyl derivative I. For this compound ten proton resonances are observed (Fig. 2), four to low field (negative spin densities) and six to high field (positive spin densities). Three resonances can be assigned immediately to the three non-equivalent sets of protons of the seven-membered ring. Four of the seven resonances attributable to the naphthalene ring exhibit resolvable nuclear spin-spin structure. The only ambiguity in assignment of the seven resonances involves the two resonances arising from protons bonded to carbon atoms adjacent to the nitrogen-substituted carbon atom and for which nuclear spin–spin splittings were not resolved. Assignment was made on the basis of a valence bond calculation for the 2-naphthylmethyl radical[8] which suggested the spin density on C-1 to be about twice that on C-3.

These chelate systems appear to be well suited to studying propagation of resonance effects through π-systems; spin density introduced onto the nitrogen atom via the nickel atom then is free to be distributed throughout the conjugated system. For example, spin densities on the terminal phenyl ring of II are about a factor of 10 less than those on the first ring. That this attenuation is due at least in part to the non-planarity of the biphenyl group is evidenced by the observation that the C-7 atom of the chelate of N,N'-di-(2-fluorenyl)-aminotroponeimine has a spin density 2.5 times greater than that of the C-4' atom of the biphenyl derivative.

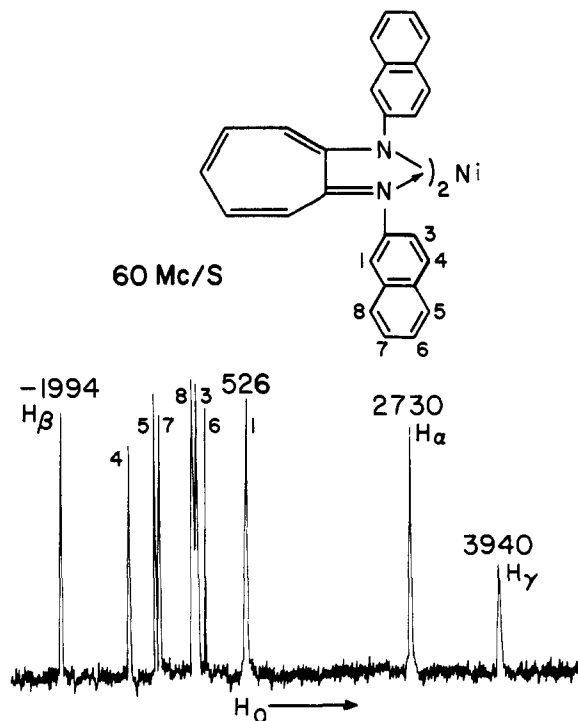(8) H. H. Dearman and H. M. McConnell, J. Chem. Phys., 33, 1877 (1960).

Fig. 2.—H[1] spectrum of the nickel(II) chelate of 1-(2-naphthylamino)-7-(2-naphthylimino)-1,3,5-cycloheptatriene in CDCl₃; spectrum internally referenced to (CH₃)₄Si.

Spin density effects are propagated through hetero atoms. This is illustrated by the azo derivative III in which appreciable spin densities are

observed 15 Å. away from the nickel atom. The –NH– group is found to be as effective as the N=N group in transmitting spin density effects. However, substitution of an oxygen atom for the N=N linkage in III reduces spin densities on the terminal phenyl ring by a factor of about five. This strong attenuating effect of the oxygen atom can also be seen in IV from comparison of the observed spin densities on the six- and seven-membered rings. A rather similar spin density distribution was observed for the phenyl ring of the analog of IV in which oxygen is replaced by sulfur. These studies clearly establish that π-electron spin densities are transmitted through hetero atoms linking conjugated systems.

The sign of the spin density of the Cγ atom of IV is positive (see I, II and III), whereas the spin density of C-1 of the phenyl ring must be negative. It would appear then that structures such as
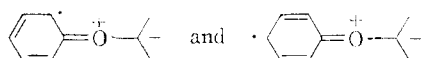
contribute significantly to the electronic structure of the molecule since they would place positive spin densities on the ortho and para carbon atoms. However, as expected from consideration of contributing valence bond structures, simple alternation of sign of the spin density distribution takes place across the group in III, where each of the

$$>C-N=N-C<$$
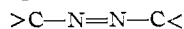
bridging nitrogen atoms contributes only a single p π electron.

Details of spin density distribution measurements and valence bond calculations on these and other aminotroponeimineates will be published shortly.

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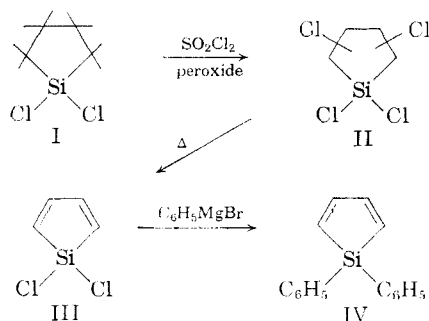   RECEIVED JULY 10, 1961

A NEW CLASS OF ORGANOSILICON COMPOUNDS. SILICON ANALOGS OF CYCLOPENTADIENE

Sir:

We are hereby reporting the first unequivocal synthesis of a series of silicon compounds which are analogs of cyclopentadiene.[1]

1,1-Dichlorosilacyclopentadiene (III) has been prepared by the reaction sequence depicted

(1) **Hexaphenysilacyclopentadiene** has been mentioned (E. H. Braye and W. Hübel, *Chem. and Ind.*, 1250 (1959)), but no details of its preparation or structure proof are given.

Treatment of silicon tetrachloride with the di-Grignard reagent of 1,4-dibromobutane produced I (b.p. 142-3°).[2] Treatment of the latter with two equivalents of sulfuryl chloride in the presence of benzoyl peroxide formed an isomeric mixture of tetrachlorosilacyclopentanes (II) (b.p. 63-70° (2.5 mm.). *Anal.*, Calcd. for $C_4H_6SiCl_4$: C, 21.42; H, 2.67; Cl, 63.39. Found: C, 21.64; H, 2.54; Cl, 63.33. When II was passed through a packed tube heated at 525-540°, 1,1-dichlorosilacyclopentadiene (III) was obtained as a colorless liquid boiling at 130° (dec.) *Anal.* Calcd. for $C_4H_4SiCl_2$: C, 31.78; H, 2.64; Cl, 47.00. Found: C, 31.94; H, 2.81; Cl, 47.09.

Compound III reacted with phenylmagnesium bromide to form 1,1-diphenylsilacyclopentadiene (IV) m.p. 55-56°. (*Anal.* Calcd. for $C_{16}H_{14}Si$: C, 82.05; H, 5.98. Found: C, 81.91; H, 5.99) and with lithium aluminum hydride to form silacyclopentadiene (b.p. 60-62°). The infrared spectrum of IV showed typical Si C_6H_5 absorption at 1590, 1490, 1430, and 1120 cm.$^{-1}$. Compound IV formed an adduct with hexachlorocyclopentadiene, m.p. 41-42° [*Anal.* Calcd. for $C_{21}H_{14}SiCl_6$: C, 49.70; H, 2.76; Cl, 42.01. Found: C, 49.67; H, 3.09; Cl, 42.20] and tetracyanoethylene.

The way is now clearly open for an investigation of the stability of the anion of silacyclopentadiene and concomitant implications with regard to aromaticity and the Hückel rule.[3]

It is also obvious that a route may now be opened for the formation of "sandwich" structures of the ferrocene type from silacyclopentadiene. Experiments are already underway in our Laboratory to investigate these points. A broad research program is envisioned.

(2) R. J. West, *J. Am. Chem. Soc.*, **76**, 6012 (1954).
(3) E. Hückel, *Z. Elektrochem.*, **43**, 752, 827 (1937).

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                    RECEIVED AUGUST 7, 1961

THE ROLE OF ACETATE, MALONATE AND SUCCINATE IN THE BIOSYNTHESIS OF CAROLIC ACID[1]

Sir:

The mold tetronic acids have a unique structural relationship both to ascorbic acid and to fatty acids. Lybing and Reio[2] indicated that part of the carolic acid molecule was derived by the "head to tail" condensation of acetate, commonly observed in mold metabolites. Evidence that this polyacetic condensation actually involves malonate is now reported; a role for a C_4 dicarboxylic acid in carolic acid formation also has been demonstrated.

Carolic acid was isolated[3] from cultures of *Penicillium charlesii* NRRL 778, grown on ordinary

(1) Supported in part by a grant from the National Science Foundation.
(2) S. Lybing and L. Reio, *Acta Chem. Scand.*, **12**, 1575 (1958).
(3) P. W. Clutterbuck, W. N. Haworth, H. Raistrick, F. Reuter and M. Stacey, *Biochem. J.*, **28**, 94 (1934); P. W. Clutterbuck, H. Raistrick and F. Reuter, *ibid.*, **29**, 300 (1935).