+60.0° (benzene); Found: C, 58.7; H, 5.7; N, 8.0. The racemate, formed by recrystallization of a mixture of equal quantities of III and IV from benzene–methanol, melted at 264–265°. Partial hydrolysis of III with sodium hydroxide in aqueous dioxane yielded (−)-menthyl hydrogen 2,6,2′,6′-tetranitro-4,4′-diphenate (V), m.p. 216–218°, $[\alpha]^{31}D$ −38.6° (acetic acid); Found: N, 9.7, 9.5; neut. eq. 562. Treatment of V with thionyl chloride, followed by addition of (−)-menthol in pyridine, gave III (m.p., mixed m.p., $[\alpha]_D$). Similar treatment of the acid chloride of V with (+)-menthol afforded I, m.p. 247–248.5° (Found: C, 58.4; H, 6.3; N, 8.1). The optical activity of I was zero, as measured in pyridine and benzene solutions at 589, 578, 546 and 435 mμ.

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THE STEREOCHEMISTRY OF HYPERCONJUGATION

Sir:

The most important recent evidence for the concept of hyperconjugation has been the finding of decreased reactivity in solvolytic reactions of tertiary halides and secondary sulfonates in which β-hydrogen atoms are substituted by deuterium.[1,2] The accepted explanation has involved the decreased effectiveness of hyperconjugation (I ⟷ II) because of the greater strength of the carbon–deuterium bond. Changes in inductive effect are not involved for HD has no dipole moment. The implied analogy between hyperconjugation and elimination reactions[1] and the suggested importance of the *trans*-hydrogen in II[3] have been scrutinized by a study of the stereospecificity of the deuterium isotope effect.

$$\underset{\text{I}}{H{-}CR_2{-}\overset{\delta+}{C}R_2\cdots\overset{\delta-}{X}} \longleftrightarrow \underset{\text{II}}{H^+\ CR_2{=}CR_2\ X^-}$$

Reaction of cyclopentene oxide with lithium aluminum deuteride gave *trans*-cyclopentanol-2-*d* (III) containing 0.98 ± 0.03 atom of D per molecule, which was converted to the tosylate, m.p. 28–29°, and displaced by tetramethylammonium acetate in pure acetone to afford, after hydrolysis, *cis*-cyclopentanol-2-*d* (IV). The infrared spectra of III and IV were different in many respects and demonstrated that each deuteroalcohol was free from its epimer.

Ten exchanges of cyclopentanone with excess weakly basic deuterium oxide gave cyclopentanone-2,2,5,5-d_4, which was reduced with lithium aluminum hydride at −80° to cyclopentanol-2,2,5,5-d_4 (V) containing 4.1 ± 0.1 atoms of D per molecule. The tosylate had m.p. 28–29°.

The acetolysis rates were determined at 50° for the tosylates of III, IV, V and cyclopentanol (Table I). There is no important stereochemical effect for deuterium substitution. The tosylates of III and IV solvolyzed at closely similar rates and showed one-fourth the logarithmic rate reduction of the tetradeutero derivative.

(1) V. J. Shiner, Jr., THIS JOURNAL, **75**, 2925 (1953); **76**, 1603 (1954).

(2) E. S. Lewis and C. E. Boozer, *ibid.*, **76**, 791 (1954).

(3) G. Baddeley, *Ann. Repts. on Progress Chem.* (Chem. Soc. London), **51**, 169 (1954).

TABLE I

ACETOLYSIS RATES OF DEUTEROCYCLOPENTYL TOSYLATES

Tosylate of	10^5k (sec.$^{-1}$)[a] 50°	$\Delta\Delta F^{\neq}$ (per D), cal./mole
Cyclopentanol	4.21	..
trans-Cyclopentanol-2-*d*	3.62	99
cis-Cyclopentanol-2-*d*	3.47	125
Cyclopentanol-2,2,5,5-d_4	2.05	116

[a] 0.1*M* solutions in acetic acid, 0.117*M* in sodium acetate. Rates were run in duplicate; reproducibility was 1%.

The results are clearly inconsistent with a common interpretation of resonance structures such as II. The results are completely consistent with a molecular orbital viewpoint formulated as in Fig. 1.

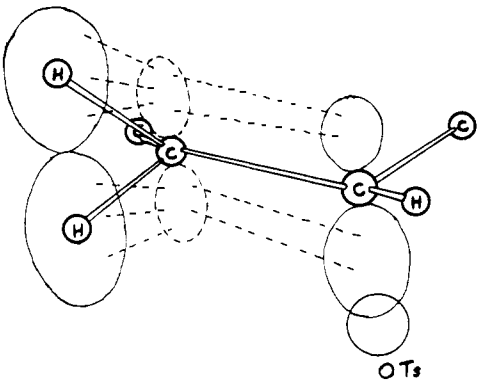

Fig. 1.—Transition state of a solvolytic reaction of cyclopentyl tosylate illustrating a molecular orbital viewpoint of hyperconjugation.

The sp^3 hybrid orbitals of the β-C–H bonds are not orthogonal to the developing p orbital at the reactive center; consequently, overlapping will occur. A molecular orbital of the proper symmetry may be constructed from the methylene hydrogen atoms which, by overlapping with the component p orbital of the β-carbon, forms a conjugated system with the developing p orbital formally analogous to that in an allyl carbonium ion. *Because the two methylene hydrogens are acting as a unit in a molecular orbital, substitution of either one by deuterium will have the same effect on the energy of the pseudo-π bond, to a close approximation.*

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THE CARCINOSTATIC ACTIVITY OF SOME 2-AMINO-1,3,4-THIADIAZOLES

Sir:

During screening of compounds for their carcinostatic activity, several 2-amino-1,3,4-thiadiazole derivatives were found to be active against several transplanted animal tumors. A representative group of the derivatives and analogs synthesized and tested are listed, along with the results obtained, in Table I. The synthesis of these particu-

lar compounds has been previously described by others.[1]

TABLE I

CARCINOSTATIC ACTIVITY OF SOME 2-AMINO-1,3,4-THIADIAZOLES

R	S91 Melanoma Daily dose, mg./kg.	S91 Melanoma % Tumor[a] inhibition	8110 Glioblastoma Daily dose, mg./kg.	8110 Glioblastoma % Tumor[a] inhibition	6C3HED Lymphosarcoma Daily dose, mg./kg.	6C3HED Lymphosarcoma % Tumor[a] inhibition
2-R-Amino-1,3,4-thiadiazole						
H	25	94	120	71	100	99
	3.75	57	100	51	50	94
CH_3	250	94	500	61		
	187	78	375	40		
C_2H_5	100	88	200	63	250	94
	15	70	150	51		
Allyl	125	89	125	62	100	28
	62.5	82	62.5	26		
Phenyl	150	0	37.5	8		
	75	0	12.5	0		
Acetyl	100	81	200	78	150	61
	75	33	100	23	75	52
2-Amino-5-R-1,3,4-thiadiazole						
OH	187	54	500	70		
			250	50		
SH	500	27	500	0	500	0
	250	0	250	0	250	0
Cl	100	87	100	10	250	87
	75	76	75	31	125	80
2-R-Amino-5-methyl-1,3,4-thiadiazole						
CH_3	300	20	300	0		
	125	0	125	15		
Allyl	250	30	250	0		
	125	0	125	0		

[a] % tumor inhibition = $100 - \frac{\text{Av. tumor weight of treated mice} \times 100}{\text{Av. tumor weight of control mice}}$

The tumors used were the S-91 melanoma of the DBA-line 1 mouse, the 8110 glioblastoma of the A mouse and the 6C3HED lymphosarcoma of the C3H mouse. These tumors were implanted into the appropriate strain of mouse and allowed to become established before treatment was started. The compounds were given in daily intraperitoneal doses at the levels indicated in Table I. The melanoma was treated for two weeks, the other tumors for one week. The tumors were then excised and weighed. The highest doses shown are approximately the maximum tolerated doses of the compounds.

From the results shown in Table I the parent compound, 2-amino-1,3,4-thiadiazole, appears to be the most active. The 2-lower alkylamino and 2-acylamino derivatives were also active and less toxic than the parent amino compound, while the 2-phenylamino derivative was inactive. In most cases substitution in the 5 position reduced the activity of the 2-amino derivatives.

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(1) (a) L. L. Bambas, "The Chemistry of Heterocyclic Compounds," Interscience Publishers, Inc., New York, N. Y., 1952; (b) M. Freund and H. P. Schwartz, *Ber.*, **29**, 2487 (1896).

THE OCCURRENCE OF DEOXY-PYRIMIDINE NUCLEOTIDES IN THE ACID-SOLUBLE EXTRACT OF THYMUS[1]

Sir:

A previous report suggesting the natural occurrence of thymidinetriphosphate and possibly other deoxy-nucleotides has appeared.[2] The recently reported[3] synthesis *in vitro* of ribo-polynucleotides from diphospho-ribonucleotides with a soluble enzyme preparation from *Azotobacter vinelandii* has drawn attention to the high-energy nucleotides as direct precursors of polynucleotides. Although Kanazir[4] has reported finding thymidylic acid in soluble extracts of *E. coli* and Schneider[5] has reported finding deoxy-pyrimidine nucleosides in rat tissue extracts, no one, to the authors' knowledge, has reported finding deoxy-nucleotides in soluble extracts of mammalian tissues. This report presents evidence for the occurrence of the mono-, di- and triphosphate derivatives of thymidine and deoxycytidine in cold perchloric acid extracts of fresh calf thymus.

The neutralized extract was chromatographed on Dowex-1 by extended gradient elution[6] with the formic acid system. A compound tentatively identified as TTP,[7] but poorly resolved from GTP and UTP, was hydrolyzed in *N* HCl and rechromatographed in the AM-F system[6] to yield TMP. Analytical data, in μM./μM. of nucleotide (amounts based on ultraviolet spectral data), were: deoxyribose,[8] 0.93; 5′-P,[6] 0.96; and total P, 0.99. On paper chromatography in three-solvent systems, the sample exhibited essentially the same R_f's as authentic TMP (Table I). Hydrolysis of this TMP at the glycosidic bond gave a compound which had an R_f identical to thymine in three solvent systems (Table II). Incomplete enzymatic hydrolysis of the TTP peak by potato apyrase[9] gave TMP and

(1) This work performed under Atomic Energy Commission Contract No. AT(11-1)-75.

(2) R. L. Potter, *Fed. Proc.*, **14**, 263 (1955).

(3) M. Grunberg-Manago and S. Ochoa, THIS JOURNAL, **77**, 3165 (1955); M. Grunberg-Manago and S. Ochoa, Abstracts of Papers Presented at the American Chemical Society Meetings, Sept. 11–16, 1955.

(4) D. Kanazir, *Biochim. et Biophys. Acta*, **13**, 589 (1954).

(5) W. C. Schneider, *J. Biol. Chem.*, **216**, 287 (1955).

(6) R. B. Hurlbert, H. Schmitz, A. Brumm and V. R. Potter, *ibid.*, **209**, 23 (1954).

(7) The following abbreviations have been used: R_f, ratio of the movement of a band to the movement of the solvent front; AM-F, ammonium formate; TMP, TDP, TTP, the mono-, di-, and triphosphates of thymidine; ATP, adenosine triphosphate; GTP, guanosine triphosphate; UTP, uridine triphosphate; CMP, CDP, CTP, D-CMP, D-CDP, D-CTP, the mono-, di-, and triphosphates of cytidine and deoxy-cytidine, respectively.

(8) S. Brody, *Acta Chem. Scand.*, **7**, 502 (1953).

(9) P. S. Kirshnan, *Arch. Biochem.*, **20**, 261 (1949).