These data are different from those found for the three-stranded helical complex between an adenine tetraribonucleotide and two long strands of polyribouridylic acid.[5] Apparently in this system, when the complex forms, it becomes a complete, rigid helix with no flexibility at the ends of the short adenine polymers.

The results presented here corroborate the theoretical analysis of the hypochromic effect. They also point out that one must be cautious in assuming that the amount of hypochromism is a valid index of helix content. For example, a partially denatured DNA sample with 50% in the form of a helix would have 50% hypochromism if the helix part of each molecule was in very long continuous segments. However, if there were on the average six optically interacting base pairs in a helical region and then an equal number of optically independent residues in a coil, the observed hypochromism would be near 35%.

We wish to thank Professor H. G. Khorana for his generous gift of deoxyribothymidylic acid oligonucleotides.[6]

(5) M. N. Lipsett, L. A. Heppel and D. F. Bradley, *Biochim. Biophys. Acta*, **41**, 175 (1960).

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GAS CHROMATOGRAPHIC BEHAVIOR OF VITAMINS D_2 AND D_3

Sir:

The detection and estimation in natural materials of individual members of the Vitamin D family is a difficult problem, particularly if more than one D vitamin is present. Since the high resolving power and high sensitivity of gas chromatographic methods may now be applied to steroid problems, the behavior of vitamins D_2 and D_3 was investigated to determine whether these substances could be distinguished from each other and from provitamins D_2 and D_3 by gas chromatographic procedures.

Vitamin D_2 gave two distinct peaks, with no evidence of decomposition on the column. For comparison purposes, the relative (to cholestane) retention times were determined on a silicone SE-30 phase (non-polar) and a neopentyl glycol succinate phase (polar) for ergosterol, lumisterol, pyrocalciferol and isopyrocalciferol. Vitamin D_2 is known to undergo a thermal cyclization reaction yielding pyrocalciferol and isopyrocalciferol, and the observed relative retention times indicated that the vitamin was transformed in the "flash heating" zone of the column into a mixture of these two compounds. This was confirmed by a comparison of the infrared and ultraviolet spectra of the initial material and the corresponding spectra for material collected after gas chromatography. The infrared spectrum changes showed loss of the terminal methylene group, and the ultraviolet spectrum changes were

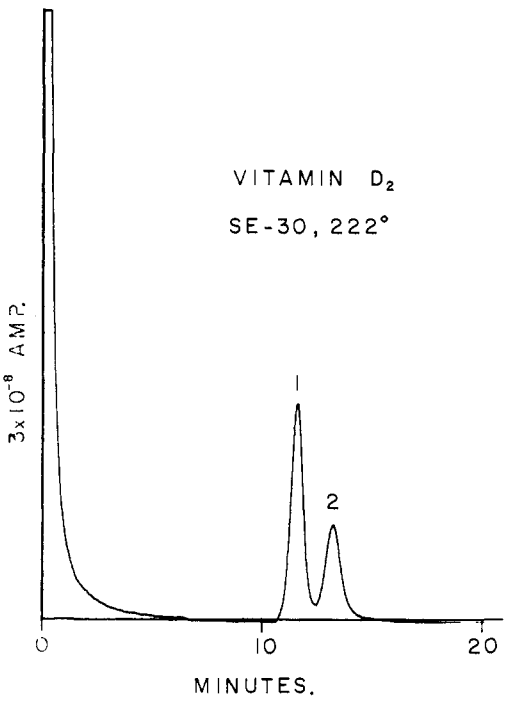

Fig. 1.—Gas chromatographic behavior of vitamin D_2, showing pyrocalciferol (1) and isopyrocalciferol (2): column, 6 ft. × 4 mm.; 0.75% SE-30 on 100–140 mesh Gas-Chrom P, 222°; 19 psi.; argon ionization detector.

those resulting from transformation of vitamin D_2 into the tetracyclic systems of the "pyro" isomers. The collected material was re-run on both phases; the observed retention times were those of pyrocalciferol and isopyrocalciferol, and no further changes were seen.

TABLE I

RELATIVE RETENTION TIMES ON NON-POLAR AND POLAR PHASES

Compound	Structure 9-H	Structure 10-CH_3	Phases SE-30[a]	Phases NGS[b]
Ergosterol	α	β	2.35	10.45
Lumisterol	β	α	1.68	6.19
Pyrocalciferol	α	α	1.83	6.10
Isopyrocalciferol	β	β	2.08	9.22
Vitamin D_2			1.84, 2.08	6.13, 9.17
7-Dehydrocholesterol	α	β	2.05	9.37
Vitamin D_3			1.66, 1.88	5.52, 8.19
Cholestane			1.00[c]	1.00[d]

[a] Column conditions: 0.75% SE-30 silicone on 100–140 mesh Gas-Chrom P; 222°; 19 p.s.i.; 6 ft. × 4 mm. col. [b] Column conditions: 0.75% neopentyl glycol succinate on 100–140 mesh Gas-Chrom P; 210°; 22 p.s.i.; 6 ft. × 3 mm. col. [c] Time, 6.2 minutes. [d] Time, 4.4 minutes.

The influence of the "flash heating" temperature on the transformation was investigated. The ratio of areas of the two peaks (pyrocalciferol: isopyrocalciferol) was compared (SE-30 phase) for three temperatures, with these results: the ratio was 1.86 at 230°, 1.78 at 267° and 1.73 at 300°. The data indicate that the ratio of products is only slightly dependent on the temperature of the cyclization reaction under these conditions.

When these methods were used for vitamin D_3 a comparable result was found. Two compounds

(ratio 1.87 at 250°) were obtained on chromatography, and a comparison of the ultraviolet and infrared spectra of the starting material and collected material indicated that a cyclization corresponding to the D_2 transformation had occurred. The observed retention times may be regarded as arising from pyrovitamin D_3 and isopyrovitamin D_3.

Although the chromatography of vitamins D_2 and D_3 is accompanied by a thermal change, it may be possible to use the resulting separation patterns for identification or estimation purposes. The "pyro" and "isopyro" compounds do not occur naturally, and the relative retention times are quite distinct from those of the provitamins. These methods might also be useful in studying irradiation mixtures.

The techniques used here were those described in previous work in steroid separations.[1]

(1) W. J. A. VandenHeuvel, C. C. Sweeley and E. C. Horning, THIS JOURNAL, **82**, 3481 (1960) (steroids); W. J. A. VandenHeuvel, C. C. Sweeley and E. C. Horning, *Biochem. Biophys. Res. Comm.*, **3**, 33 (1960) (sex hormones and bile acids); W. J. A. VandenHeuvel and E. C. Horning, *ibid.*, **3**, 356 (1960) (adrenal cortical steroid hormones); W. J. A. VandenHeuvel, C. C. Sweeley and E. C. Horning, "Separation of Steroids by Gas Chromatography," Symposium on Drugs Affecting Lipid Metabolism, Milan, Italy, June 2–4, 1960 (sterols and sterol esters); C. C. Sweeley and E. C. Horning, *Nature*, **187**, 144 (1960) (steroids); W. J. A. VandenHeuvel, E. C. Horning, Y. Sato and N. Ikekawa, *J. Org. Chem.*, in press (steroidal amines); R. K. Beerthuis and J. H. Recourt, *Nature*, **186**, 372 (1960) (sterols).

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EFFECT OF SOLVENT ON RATE OF BASE-CATALYZED PROTON ABSTRACTION FROM CARBON

Sir:

In connection with our study of electrophilic substitution at saturated carbon,[1] we observed that with potassium *tert*-butoxide as a basic catalyst, optically active 2-phenylbutane could be racemized at a much lower temperature in dimethyl sulfoxide than in *tert*-butyl alcohol as solvent. Kinetic studies have now revealed that the rates of proton abstraction from carbon by alkoxide anions can be made to vary by as much as an estimated nine powers of ten by simply a change in solvent.

Racemization of 2-methyl-3-phenylpropionitrile (I) was found to proceed at a convenient rate in mixtures of methanol and dimethyl sulfoxide with metal methoxides as bases. In other studies[2] hydrogen-deuterium exchange and racemization rates were found to be equal with this substrate in ethylene glycol and in *tert*-butyl alcohol, and these two rates for this nitrile are presumed to be equal in any solvent–base system. At concentrations of base up to 0.01 molar, the rates were found to be cleanly first order in base and in substrate, and independent of the nature of the cation of the base (lithium, sodium or potassium) over a solvent composition that ranged from pure methanol to 3% methanol–97% dimethyl sulfoxide (by weight). A single rate determination was made at 1.5% methanol–97% dimethyl sulfoxide (by weight) with potassium methoxide as base. Rates at 76.5% and higher dimethyl sulfoxide concentration were obtained at 25° by direct polarimetric measurement, whereas at lower concentrations, rates were determined at two higher temperatures with the ampoule technique, and rate constants were extrapolated to 25°. For purposes of comparison, a rate run was also conducted at 25° in *tert*-butyl alcohol with 0.0016 *M* potassium *tert*-butoxide as base. Table I records the relative rate factors.

TABLE I

RELATIVE RATES OF RACEMIZATION OF NITRILE (I)

Solvent (% by weight)	Base *M* is Li, Na, K	Rel. rates (25°)
100 CH_3OH–0(CH_3)$_2SO$	CH_3OM	1
75 CH_3OH–25 (CH_3)$_2SO$	CH_3ONa	3.2×10^1
49.6 CH_3OH–50.4 (CH_3)$_2SO$	CH_3ONa	1.6×10^2
23.5 CH_3OH–76.5 (CH_3)$_2SO$	CH_3OLi	4.9×10^3
10 CH_3OH–90 (CH_3)$_2SO$	CH_3OM	1.3×10^5
3 CH_3OH–97 (CH_3)$_2SO$	CH_3OM	1.4×10^6
1.5 CH_3OH–98.5 (CH_3)$_2SO$	CH_3OK	$\sim 1.05 \times 10^7$
0 CH_3OH–100 (CH_3)$_2SO$	CH_3OK	$\approx 10^9$ (estd.)
100 (CH_3)$_3COH$	(CH_3)$_3COK$	4.1×10^6

With optically active 1-phenylmethoxyethane as substrate, a similar but less extensive comparison was made between the rates of racemization with potassium *tert*-butoxide as base in dimethyl sulfoxide on the one hand, and *tert*-butyl alcohol on the other. In the latter solvent, the rate was measured at 173°, and in the former, at 49° and 75°. Extrapolation of the rate constant in dimethyl sulfoxide to 173° gave a value $>10^6$ greater than the value of the rate constant in *tert*-butyl alcohol. The rate of hydrogen–deuterium exchange is at least a factor of ten higher than the rate of racemization for this system in *tert*-butyl alcohol, but the two rates are equal in dimethyl sulfoxide.[3] Thus the rate of proton removal from 1-phenylmethoxyethane by potassium *tert*-butoxide is $\sim 10^7$ greater in dimethyl sulfoxide than in *tert*-butyl alcohol. The combined data represent a spread of eleven powers of ten between the rates of proton removal from carbon by methoxide ion in methanol and by potassium *tert*-butoxide in dimethyl sulfoxide. The vastly enhanced activity of alkoxide ions in dimethyl sulfoxide over their activity in alcohols is attributed to the absence of alkoxide-solvent hydrogen bonds in dimethyl sulfoxide which are present in the hydroxylic solvents. Preliminary experiments indicate that tetramethylene sulfone as solvent[4] also enhances the catalytic activity of alkoxide ions, but less than dimethyl sulfoxide.

These observations suggest that the rates of many base-catalyzed reactions can be enhanced greatly by substitution of dimethyl sulfoxide for the usual hydroxylic solvents. A dramatic ex-

(1) D. J. Cram, J. L. Mateos, F. Hauck, A. Langemann, K. R. Kopecky, W. D. Nielsen and J. Allinger, THIS JOURNAL, **81**, 5774 (1959).

(2) D. J. Cram, W. D. Nielsen and B. Rickborn, *ibid.*, **82**, 6415 (1960).

(3) D. J. Cram, C. A. Kingsbury and B. Rickborn, *ibid.*, **81**, 5835 (1959).

(4) See C. H. Langford and R. L. Burwell, Jr., *ibid.*, **82**, 1503 (1960).