The ester was half hydrolyzed during two minutes at 100° in 1.0 *N* nitric acid to give only one P^{32}-labeled product which was identified as glycerophosphate by paper chromatography and electrophoresis. The unknown was anionic in the *p*H range 2–12 and readily separable from the known glycerophosphoryl esters of the phospholipids.

Scenedesmus-C^{14} was cultured and the unknown was isolated and identified with the P^{32}-labeled compound. Acid hydrolysis produced equal activities of glycerophosphate and glycerol which was identified by its R_f values and by quantitative periodate oxidation to formaldehyde and formate. Periodate oxidation of the ester indicated an α,α'-structure. Synthetic α,α'-diglycerophosphate[3] was prepared and found inseparable from the radioactive unknown by paper chromatography and electrophoresis. Its identical hydrolysis rate was determined by neutron activation chromatography of the products.

Tetraacetyl bis-(L-α-glyceryl) phosphates have been synthesized by Baer.[4] Polyglycerophosphate structures in several lipids have been reported[5,6,7] but diglycerophosphate has not been identified previously. Its symmetry, simplicity and acid lability to give the usual products of lipid hydrolysis apparently have postponed its identification as a metabolite. Its occurrence in the glyceryl phosphatides will be reported.

We are indebted to Dr. R. J. Suhadolnik for invaluable assistance in the early stages of the identification. This work was supported by the National Science Foundation, the Atomic Energy Commission and the Pennsylvania Agricultural Experiment Station.

(3) E. Fischer and E. Pfähler, *Ber.*, **53**, 1606 (1920).

(4) E. Baer, *J. Biol. Chem.*, **198**, 853 (1952).

(5) M. C. Pangborn, *ibid.*, **168**, 351 (1947).

(6) J. M. McKibbin and W. E. Taylor, *ibid.*, **198**, 853 (1952).

(7) P. Fleury, *Bull. Soc. Chim. Biol.*, **30**, 521 (1948).

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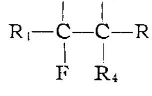
NUCLEAR MAGNETIC RESONANCE SPECTRA. HINDERED ROTATION AND MOLECULAR ASYMMETRY[1]

Sir:

The elegant and unequivocal demonstration of non-equivalent fluorine atoms in certain *gem*-difluoro compounds of type I with the aid of their nuclear magnetic resonance spectra has been interpreted as indicating restricted rotation about the central C–C bond of I.[2]

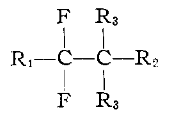


I

$$R_1-\underset{F}{\overset{F}{C}}-\underset{R_4}{\overset{R_2}{C}}-R_3$$

	R_1	R_2	R_3	R_4
Ia	Br	H	Cl	Br
Ib	Br	F	Cl	Br
Ic	Br	H	Br	C_6H_5
Id	Cl	H	Cl	C_6H_5

Significantly, spectra for the "freely rotating" substances with "equivalent" *gem*-fluorines were not produced at temperatures up to 200°.[2] This behavior suggested to us the possibility that aliphatic compounds of type II might be resolvable into reasonably stable optical antipodes at room temperature since the barriers for rotation about the central C–C bond of II a-c should be substantially greater than for Ia-d.



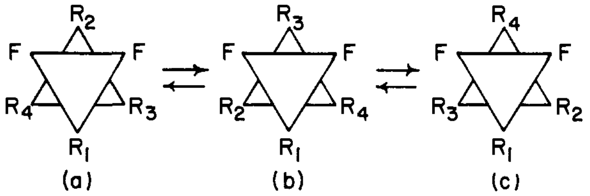
II

$$R_1-\underset{F}{\overset{F}{C}}-\underset{R_3}{\overset{R_3}{C}}-R_2$$

	R_1	R_2	R_3
IIa	Br	F	Br
IIb	Br	Br	Cl
IIc	Br	C_6H_5	Br

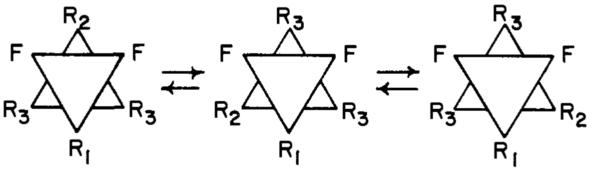
This expectation was not realized. The nuclear magnetic resonance spectra of IIa-c showed *equivalent gem*-fluorines down to -30°. Thus IIa shows doublet CF_2 and triplet CF absorptions separated by 440 c.p.s. with J equal to 18–19 c.p.s. The results indicate that there is rapid rotation at room temperature about the central C–C bond in IIa-c and, by inference, in Ia-d also.[3]

The solution to the apparent paradox is that the chemical shift between *gem*-groups as in compounds like I is not necessarily averaged by rapid rotation *unless the residence times of the molecule in each of the various rotational conformations (III) are equal.* The *gem*-group does not have equivalent atoms in any of the configurations IIIa, b or c. Therefore unless the residence times in each configuration are equal, the chemical shift difference between the *gem*-fluorines will not average out in the general case.[4] Since the relative residence times will be temperature dependent, the apparent degree of non-equivalence of the fluorines will vary with temperature as is observed.[2]



(a) (b) (c)

III



(a) (b) (c)

IV

With more symmetrical compounds like II, rapid rotation at $> -30^\circ$ will average the chemical shifts of the *gem*-difluorines since the residence times (and populations) for the two configurations with non-equivalent fluorines (IVb,c) must be

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(1) Supported in part by the Office of Naval Research.

(2) J. J. Drysdale and W. D. Phillips, THIS JOURNAL, **79**, 319 (1957).

(3) Drs. W. D. Phillips and J. J. Drysdale have informed us that they have independently made similar observations on fluorine compounds of related structure.

(4) In some situations, the spin–spin coupling between the non-equivalent fluorines, or other similarly located atoms, may be so large as to swamp out the chemical shift and give to all intents and purposes a single resonance line.

equal since they are optical antipodes. IVa may have a very different residence time but has equivalent fluorines. At low temperatures in CS_2 solution, IIb and IIc show evidence of slow rotation about the C–C bond.[3] Thus at $-80°$, IIb appears to "freeze" to about a 1.4:1 mixture (analysis by line heights) of IVa (identifiable by its single resonance) and the *d,l*-pair IVb,c (identifiable by its quartet of resonance lines). With IIc, the rotational isomer IVa appears only to be present to the extent of about 10% at $-80°$.

Similar observations have been made for proton spectra of a few[4] compounds similar to I and II with $-CH_2-$ in place of $-CF_2-$. Compounds related to I like methyl 2,3-dibromo-2-methylpropanoate show "abnormal" spin–spin splittings for the $-CH_2-$ absorptions while those related to II like 1,2-dibromo-2-methylpropane give normal patterns; *cf.* Fig. 1. With substances like these, rotation about the C–C single bonds are unquestionably rapid.

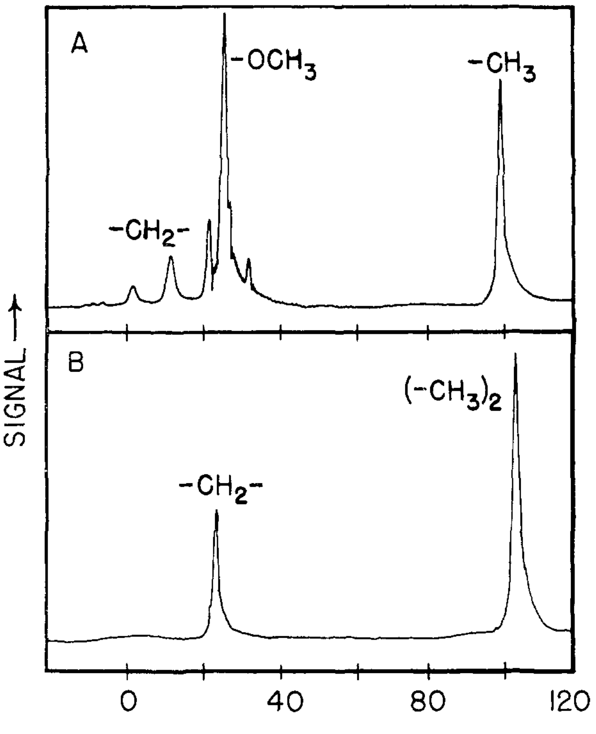

Fig. 1.—Pure liquid n.m.r. spectra of A, methyl 2,3-dibromo-2-methylpropanoate (J for coupling between *gem*-hydrogens at 3-position ~10–11 c.p.s.); B, 1,2-dibromo-2-methylpropane, taken with Varian Associates High Resolution Nuclear Magnetic Resonance Spectrometer, 12-in. magnet equipped with Super Stabilizer and at room temperature and 40 mc. Zero of reference scale was water absorption.

The importance of these observations is several fold. First, they establish beyond doubt that the residence times and populations for the various possible rotational configurations of compounds like I are not equal; second, with the aid of the reasoning used previously,[2] it becomes possible in principle to estimate, and perhaps determine, the population of the several configurations even though these are being rapidly interconverted. Third, populations of configurations may be measured directly for favorable cases by "freezing in" at low temperatures. Finally, a new and extraordinarily simple means is provided for possible detection of a carbon attached to a group without a plane of symmetry by observation of nuclear magnetic resonance spin–spin couplings without recourse to resolution into optical antipodes.[5]

(5) The method does not strictly provide a means for locating asymmetric atoms. Thus, 2-methyl-2-nitropropanediol shows nonequivalent methylene hydrogens although it is not capable of resolution into optical antipodes. However, each of the methylenes is attached to a group which has no plane of symmetry.

(6) Arthur Amos Noyes Fellow, 1956–1957.

CONTRIBUTION NO. 2211
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NEW LINES IN THE X-RAY DIFFRACTION PATTERN OF ORTHORHOMBIC SULFUR. "AMORPHOUS" (μ)-SULFUR

Sir:

In connection with another problem[1] we had occasion to purify some sulfur by a recommended[2–4] method.[5] On comparing the X-ray diffraction pattern with the ASTM card file[6] for orthorhombic sulfur, we were surprised to find several extra lines in our diffraction pattern.[7] Since different radiation sources were used[8] one might expect differences in relative intensity values but not in the number and positions of diffraction peaks. We found later that the sulfur we purified was not completely soluble in carbon disulfide and thus considered the possibility that the new lines we observed might be due to the presence of this insoluble sulfur.[9] Thus we determined the diffraction pattern

(1) A. G. Pinkus and J. S. Kim, *Can. J. Chem.*, accepted for publication, 1957.

(2) "Gmelin's Handbuch der anorganischen Chemie," System-Nummer 9, Schwefel, Teil A, Lieferung 3, 8 Auflage, Verlag Chemie, G.m.b.H., Weinheim/Bergstrasse, 1953, pp. 511, 512.

(3) "IUPAC Münster Colloquium on Silicon-Sulphur-Phosphates," Verlag Chemie, G.m.b.H., Weinheim/Bergstrasse, 1955, p. 82.

(4) W. N. Tuller, ed., "The Sulphur Data Book," McGraw-Hill Book Co., New York, N. Y., 1954, p. 12.

(5) R. F. Bacon and R. Fanelli, *Ind. Eng. Chem.*, **34**, 1043 (1942).

(6) ASTM X-Ray Powder Data File, Special Technical Publication No. 48-F, 1957.

(7) Our data have been deposited with Dr. G. W. Brindley, Editor, "ASTM X-Ray Powder Data File," College of Mineral Industries, Pennsylvania State University, University Park, Pa., and list the d values and intensities of reflection.

(8) The ASTM samples were run with Mo$K\alpha$ radiation and film whereas we used Cu $K\alpha$ radiation and a recording North American Philips X-ray diffractometer.

(9) The insoluble sulfur as determined by repeated extraction with carbon disulfide until there was no further loss in weight (within 0.2 mg.) was 3.25%. This is close to the equilibrium value of 3.75% at 121° listed in J. W. Mellor, "A Comprehensive Treatise on Inorganic and Theoretical Chemistry," Vol. X, Longmans, Green and Co., New York, N. Y., 1930, p. 47. Following a suggestion in a letter from Dr. Fanelli that the amount of insoluble sulfur could be reduced by heating at 125° overnight followed by slow cooling, the amount of insoluble sulfur was reduced to 2.49% after one such treatment using a ten-hour cooling period (heating mantle control). After the work in this paper was completed, a paper appeared (N. H. Hartshorne and M. Thackray, *J. Chem. Soc.*, 2122 (1957)) in which the amount of insoluble sulfur was reduced by "annealing at 100° for two days" (see also the Mellor reference on this point).