8.87), which was isomerized with alkali. The resulting iso-compound, without purification, was pyrolyzed giving a small yield of 4-chloro-7-hydroxyphthalide, m.p. 155–157°, which was identified by comparison with a known sample prepared by an unambiguous synthesis reported by Boothe, *et al.*, in an accompanying communication.[10]

(10) J. H. Boothe, A. Green, J. P. Petisi, R. G. Wilkinson and C. W. Waller, THIS JOURNAL, **79**, 4564 (1957).

RESEARCH DIVISION — J. S. WEBB
ORGANIC CHEMICAL — R. W. BROSCHARD
RESEARCH SECTION — D. B. COSULICH
AMERICAN CYANAMID COMPANY — W. J. STEIN
PEARL RIVER, NEW YORK — C. F. WOLF

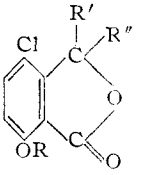
RECEIVED AUGUST 1, 1957

DEMETHYLTETRACYCLINES. SYNTHESIS OF A DEGRADATION PRODUCT

Sir:

In an accompanying communication[1] there is described a new series of antibiotics closely related to the tetracyclines, both in antibacterial activity and in structure. In a second accompanying communication[2] evidence has been presented that these new antibiotics differ from the parent compounds only in that they lack a methyl group at the 6-position of the tetracycline nucleus.

We now wish to report the synthesis of a degradation product which proves that the arrangement of substituents in the D ring and in portions of the C ring of demethylchlorotetracycline is the same as in chlorotetracycline except for the C-6 methyl group. This compound is 4-chloro-7-hydroxyphthalide (R = R′ = R″ = H) which is analogous to 4-chloro-7-hydroxy-3-methylphthalide (R = R′ = H, R″ = CH_3), obtained by the same degradative route from chlorotetracycline.[3]



The starting point for the synthesis is 4-chloro-3-hydroxy-7-methoxy-3-methylphthalide (R = R′ = CH_3, R″ = OH) whose synthesis has already been reported from this laboratory.[4] This compound was oxidized with potassium permanganate in 0.5 *N* sodium hydroxide at 90° for one hour to yield the 4-chloro-3-hydroxy-7-methoxyphthalide-3-carboxylic acid (R = CH_3, R′ = OH, R″ = COOH) in 67% yield. This compound has been isolated as a degradation product of chlorotetracycline and was named there as the tautomeric keto-acid, 6-chloro-3-methoxyphthalonic acid.[5] The reduction of this compound with sodium borohydride in *N* sodium hydroxide yielded 4-chloro-7-methoxyphthalide-3-carboxylic acid (R = CH_3, R′ = H, R″ = COOH) in 90% yield; m.p. 175–176° with effervescence; $\lambda_{max}^{0.1\,N\,HCl}$ 216 mμ (ε 32,200); 240 mμ (ε 8,240); 313 mμ (ε 5,220). $\lambda_{max}^{0.1\,N\,NaOH}$ (after standing one hour)[6] 214 mμ (ε 31,500); 285 mμ (ε 2,190).

Anal. Calcd. for $C_{10}H_7O_5Cl$: C, 49.5; H, 2.9; Cl, 14.6. Found: C, 49.5; H, 3.2; Cl, 14.8.

The phthalidecarboxylic acid was then decarboxylated by heating 5–10 minutes just above its melting point to yield 4-chloro-7-methoxy-phthalide (R = CH_3, R′ = R″ = H) which was sublimed at 175° (760 mm.); yield, 70%; m.p. 167–168° $\lambda_{max}^{0.1\,N\,HCl}$ 215 mμ (ε 37,300); 236 mμ (ε 8,830); 308 mμ (ε 4,560); $\lambda_{max}^{0.1\,N\,NaOH}$ (after standing one hour)[6] 286 mμ (ε 2,190).

Anal. Calcd. for $C_9H_7O_3Cl$: C, 54.4; H, 3.6; Cl, 17.9. Found: C, 54.8; H, 3.7; Cl, 17.7.

The methyl ether was cleaved by refluxing in 48% hydrobromic acid for 2.5 hours. The product, 4-chloro-7-hydroxyphthalide (R = R′ = R″ = H), crystallized from the hydrobromic acid on cooling in 70% yield and was then sublimed at 100° (15–20 mm.). The m.p. was 158–159° and there was no depression upon admixture with the degradation product.[2] The ultraviolet and infrared spectra were identical; $\lambda_{max}^{0.1\,N\,HCl}$ 235 mμ (ε 8,400); 308 mμ (ε 4,150); $\lambda_{max}^{0.1\,N\,NaOH}$ 254 mμ (ε 8,400); 343 mμ (ε 6,180).

Anal. Calcd. for $C_8H_5O_3Cl$: C, 52.1; H, 2.7; Cl, 19.2. Found: C, 52.2; H, 3.2; Cl, 19.0.

(1) J. R. D. McCormick, N. O. Sjolander, U. Hirsch, E. R. Jensen and A. P. Doerschuk, THIS JOURNAL, **79**, 4561 (1957).

(2) J. S. Webb, R. W. Broschard, D. B. Cosulich, W. J. Stein, and C. F. Wolf, *ibid.*, **79**, 4563 (1957).

(3) C. R. Stephens, L. H. Conover, R. Pasternack, F. A. Hochstein, W. T. Moreland, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.*, **76**, 3568 (1954).

(4) J. H. Boothe, S. Kushner, J. P. Petisi and J. H. Williams, *ibid.*, **75**, 3261 (1953).

(5) B. L. Hutchings, C. W. Waller, S. Gordon, R. W. Broschard, C. F. Wolf, A. A. Goldman and J. H. Williams, *ibid.*, **74**, 3710 (1952). For a discussion of and references to this type of tautomerism see ref. 4.

(6) Upon standing in 0.1 *N* sodium hydroxide for an hour or less the long wave length absorption maximum undergoes a hypsochromic shift which is reversible by acidification. This is assumed to be attributable to the opening and closing of the lactone ring and will be dealt with in more detail in a subsequent publication.

RESEARCH DIVISION — J. H. BOOTHE
ORGANIC CHEMICAL — A. GREEN
RESEARCH SECTION — J. P. PETISI
AMERICAN CYANAMID COMPANY — R. G. WILKINSON
PEARL RIVER, NEW YORK — C. W. WALLER

RECEIVED AUGUST 1, 1957

α,α′-DIGLYCEROPHOSPHATE IN PLANTS

Sir:

We have observed a P^{32}-labeled compound in hydrolysates of *Scendesmus* phosphatides which contained more than a third of the lipid phosphorus. The same phosphate ester also possessed as much as 90% of the alcohol-soluble non-lipid phosphorus of *Scenedesmus* cultured at low light intensity in media containing P^{32}. The cellular concentration of the ester, calculated from its P^{32} activity and the nutrient specific activity, was as high as 10^{-3} *M*. The same compound in lower concentrations occurred in the only two species of higher plants (clover) tested. It was isolated by chromatography on Whatman No. 1 paper with R_f = 0.36 in phenol–water and R_f = 0.11 in butanol–propionic acid–water.[1] These R_f values correspond to those recorded by Dawson[2] for an unknown in rat liver extracts.

(1) A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, THIS JOURNAL, **72**, 1710 (1950).

(2) R. M. C. Dawson, *Biochim. et Biophys. Acta*, **14**, 374 (1954).

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).

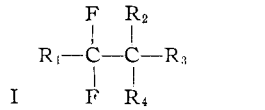
DEPARTMENT OF AGRICULTURAL AND BIOLOGICAL CHEMISTRY
PENNSYLVANIA STATE UNIVERSITY
UNIVERSITY PARK, PENNSYLVANIA
B. MARUO
A. A. BENSON

RECEIVED JULY 3, 1957

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

Sir:

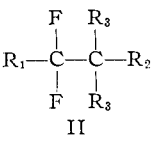
The elegant and unequivocal demonstration of non-equivalent fluorine atoms in certain *gem*-difluoro compounds of type I with the aid of their nuclear magnetic resonance spectra has been interpreted as indicating restricted rotation about the central C–C bond of I.[2]



	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

I

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.

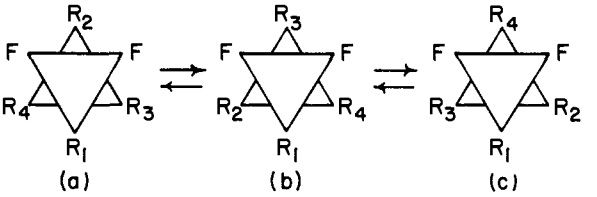


	R_1	R_2	R_3
IIa	Br	F	Br
IIb	Br	Br	Cl
IIc	Br	C_6H_5	Br

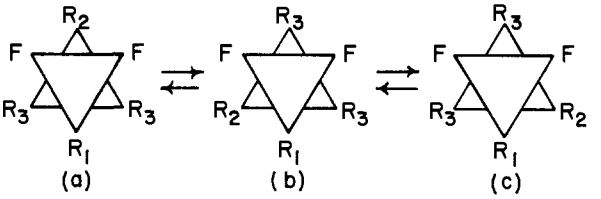
II

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



III



IV

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]

With more symmetrical compounds like II, rapid rotation at > −30° 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

(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.