

Native, Bjorkman, and Klason preparations had the lowest spin concentration; alkali and fungal preparations showed a 5–10-fold greater free-radical content. Inasmuch as the latter specimens have undergone some demethylation during their preparation, it is possible that *ortho*-quinoid or quinonemethide groups have been formed.⁵ These could accommodate a stable free radical to a greater extent than the native and acid preparations.

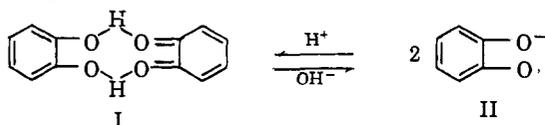
When certain lignin samples were converted to their sodium salts (by the addition of absolute ethanol to an aqueous alkaline solution of lignin), a nearly 100-fold increase in spin concentration was observed (native spruce,^{6a} yellow pine Kraft, and Indulin AT,^{6b} Table II). When these salts were acidified, the recovered products had regained their original low spin content. This behavior was not significantly altered when the salt formation process was carried out under nitrogen. Klason (sulfuric acid) samples did not yield recoverable sodium salts.

TABLE II
FREE-RADICAL CONTENT OF LIGNIN DERIVATIVES^a

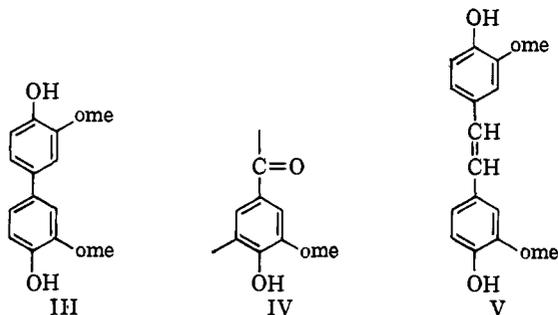
Sample	Un-treated	Sodium salt	Acidified salt	NaBH ₄ reduced	Na salt of NaBH ₄ reduced
Brauns native spruce	0.5	50	0.5	1.1	8.4
Bjorkman spruce	1.0	15			
Kraft yellow pine	3.0	100–300	3.0	1.3	22
Kraft-treated native spruce	4.0	70			
Indulin AT	3.0	72			

^a All values multiplied times 10¹⁷. Spin concentrations determined as in Table I.

A large and reversible change in spin concentration, mediated by change in pH alone, is a characteristic of quinhydrone-type systems,⁷ as indicated by the following reaction.



Other semiquinone radical-ion precursors which could form quinhydrones and which would still be consistent with well established lignin formulations are III, IV, and V.



Further support for a quinhydrone model comes from reduction experiments. Sodium borohydride, which reduces carbonyl and quinoid groups, has very little effect on the original content of the lignin,

(5) (a) C. B. Purves, N. Levitan, and N. S. Thompson, *Paper Pulp Mag.* (Canada), **56**, 117 (1955); (b) E. Adler, "Fourth International Congress of Biochemistry," Vol. II, Pergamon Press, New York, N. Y., 1959, p. 137; (c) K. Freudenberg, *ibid.*, p. 121.

(6) (a) Native spruce lignin, when subjected to Kraft pulping conditions, gave a product which exhibited the same paramagnetic properties as a commercial Kraft lignin; (b) kindly provided by the West Virginia Pulp and Paper Co., Charleston, S. C.

(7) Y. Matsunaga, *Can. J. Chem.*, **38**, 1172 (1960).

perhaps because of a balancing off of radical formation and destruction. However, it does decrease the ability of the macromolecule to form radical-ions in base (Table II), a property which is consistent with the loss of quinoid character.

Using the convention of lignin chemists, we have estimated that the semiquinone radical-ion concentration (after conversion to the salt) in native lignin is 0.0017 radical/OCH₃; for Kraft lignin the range is 0.003–0.01 radical/OCH₃. These data can also be expressed in terms of spins/molecule. For native lignin, the value is 0.01; for Kraft lignin, a range of 0.08 to 0.25 is calculated. These yields are in the range expected in view of studies with model compounds.⁷

Adler and Marton⁸ have detected as little as 0.01 carbonyl/OCH₃ by the use of ultraviolet spectrophotometry. Our results indicate a quinoid carbonyl content considerably below this value and also demonstrate the usefulness of e.p.r. spectrometry for detecting minor contributions to the structure of this macromolecule.

It appears, then, that lignin and various lignin preparations have low concentrations of organic free radicals, which are apparently stabilized by delocalization of the unpaired electron or shielded by the macromolecular network. A large number of new radical centers can be created by basification, indicating the presence of a diamagnetic quinhydrone species. The exact nature of this quinhydrone, plus structural information about related features of the lignin macromolecule, may be elucidated by causing specific chemical changes to take place and monitoring these changes by e.p.r. spectrometry. Such studies are now under way in this Laboratory.

(8) E. Adler and J. Marton, *Acta Chem. Scand.*, **15**, 370 (1961).

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Hydrogen Exchange at Methine and C-10 Positions in Chlorophyll¹

Sir:

In previous reports we have shown that both chlorophylls *a* and *b* will exchange at least one hydrogen atom with methanol in carbon tetrachloride solution,² and, from n.m.r. measurements, that the δ hydrogen exchanges with an excess of methanol in 48 hr.³ These observations are correct, but in the course of further work, we became aware that our conclusions about the relative lability of the δ and C-10 (I) protons required re-examination. Since there is considerable current interest in the possible role in photosynthesis of exchangeable hydrogen in chlorophyll, we believe it desirable to communicate our most recent results at this time.

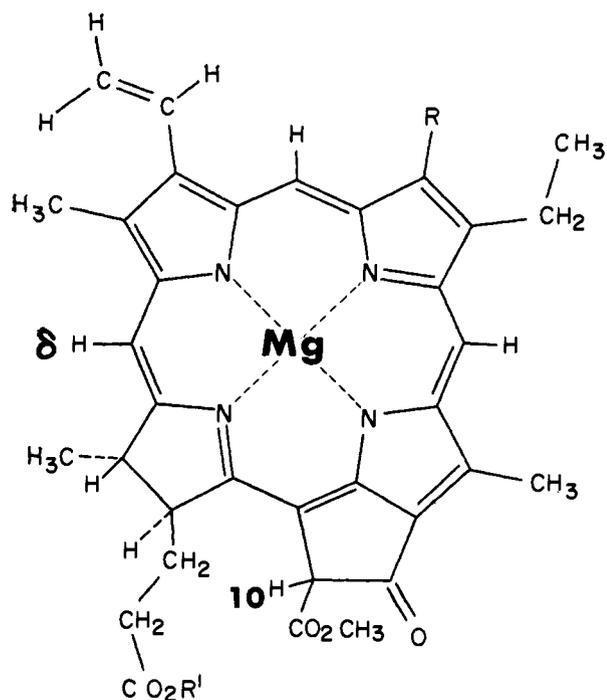
The conclusion that the δ position is more labile than the C-10 was based on n.m.r. measurements in CDCl₃ solutions very carefully freed from the CH₃OD used for the exchange. At the time this work was performed we were not aware of the remarkable effects of concentration and solvent polarity on the n.m.r. spectra of chlorophyll solutions.⁴ It is now known that in non-

(1) This work performed under the auspices of the U. S. Atomic Energy Commission.

(2) J. J. Katz, M. R. Thomas, H. L. Crespi, and H. H. Strain, *J. Am. Chem. Soc.*, **83**, 4180 (1961).

(3) J. J. Katz, M. R. Thomas, and H. H. Strain, *ibid.*, **84**, 3587 (1962).

(4) G. L. Closs, J. J. Katz, F. C. Pennington, M. R. Thomas, and H. H. Strain, *ibid.*, **85**, 3809 (1963).



polar solvents, such as pure CDCl_3 , chlorophyll is aggregated, and as a consequence the signal of the C-10 hydrogen is broadened and shifted to an extent which makes detection difficult, even in deuteriochlorophyll.⁴ Mathewson, Richards, and Rapoport,⁵ who have studied hydrogen exchange in chlorophyll, *Chlorobium* chlorophyll 660, and bacteriochlorophyll, make no mention of exchange at C-10 and report exchange only at the δ positions. Because conditions for observing the C-10 proton resonance can now be specified, we have been able to show that both the C-10 and δ protons are exchangeable, with exchange at C-10 much the faster.

The exchange with CD_3OD was studied in tetrahydrofuran, CCl_4 , and CDCl_3 . Exchange of the δ and C-10 protons is qualitatively the same in these three solvents. Tetrahydrofuran is particularly convenient, since the state of aggregation of the chlorophyll and the chemical shift of the C-10 proton vary less than is the case in CDCl_3 or CCl_4 . The results are shown in Table I. The "rate constant" for exchange of the other protons in chlorophyll and pheophytin is apparently smaller than 10^{-9} under these conditions.

TABLE I
PSEUDO-FIRST-ORDER RATE CONSTANTS FOR HYDROGEN EXCHANGE AT C-10 AND δ POSITIONS IN TETRAHYDROFURAN SOLUTIONS AT 38°C ^a

Compd.	Concn., ^b moles/l.	k^c for C-10 exchange, sec. ⁻¹	k^c for δ exchange, sec. ⁻¹	$k_{\text{C-10}}/k_{\delta}$
Chlorophyll <i>a</i>	0.185	3×10^{-4}	2×10^{-6}	150
Chlorophyll <i>b</i>	0.178	2×10^{-4}	5×10^{-6}	40
Pheophytin <i>a</i>	0.096	$>1 \times 10^{-3}$	$<10^{-9}$	$>10^6$

^a The half-time of the exchange in seconds was obtained by integration of Varian A-60 n.m.r. spectra. ^b CD_3OD concentration in all cases, 9.1 moles/l. ^c k is calculated as a pseudo-first-order rate constant from $k = \ln 2/t_{1/2}$.

The pseudo-first-order rate constants for exchange at the δ position in chlorophylls *a* and *b* have relative rates that are quite comparable with those reported by Mathewson, *et al.*⁵ Exchange at the C-10 position was in every case at least two orders of magnitude

(5) J. H. Mathewson, W. R. Richards, and H. Rapoport, *Biochem. Biophys. Res. Commun.*, **13**, 1 (1963).

faster than that of the δ and supports the chemical considerations which indicated that active hydrogen in chlorophyll was located at C-10.⁶

That exchange at the δ position in pheophytin under the conditions above is much slower than in the magnesium-containing chlorophyll derivatives reflects the effect of the central magnesium atom on the localization energy at the methine bridge. On the other hand, the exchange at C-10 in pheophytin was evidently faster than in the chlorophyll derivatives and is comparable to the rate of exchange in 2-carbomethoxycyclopentanone.

The preparation of a pyro series of chlorophyll derivatives, in which the carbomethoxy group at C-10 has been replaced by a hydrogen atom,⁷ makes it possible to compare the behavior of the δ and C-10 positions in an interesting way. Whereas the δ hydrogen in pyrochlorophyll *a* or methyl pyrochlorophyllide *a* undergoes facile exchange, the two C-10 hydrogen atoms in the pyrochlorophyllide show no measurable exchange in 2.5 months in an 11% $\text{CD}_3\text{OD}-\text{CDCl}_3$ mixture. On the other hand, when pyropheophytin *a* is heated with a mixture of pyridine and D_2O , the C-10 protons exchange and the δ position is scarcely affected. Exchange at C-10 in chlorophyll will be facilitated by either acids or bases, but exchange at the δ position probably proceeds primarily by electrophilic substitution.

(6) (a) H. Fischer and S. Goebel, *Ann.*, **522**, 168 (1936); (b) S. Aronoff, *Encyclopedia Plant Physiol.*, **5**, 234 (1960); (c) W. Vishniac and I. A. Rose, *Nature*, **182**, 1089 (1958).

(7) F. C. Pennington, H. H. Strain, W. A. Svec, and J. J. Katz, to be published.

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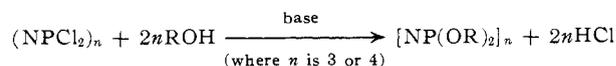
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New Reactions of Phosphonitrilic Chloride Trimer. Substitution and Cleavage Reactions with Catechol and Triethylamine¹

Sir:

Cyclic trimeric and tetrameric phosphonitrilic chlorides react with alcohols or phenols in the presence of base, or with their alkali metal salts, to yield alkyl or aryl phosphonitrilates.²⁻⁸ Dihydroxy compounds have



also been employed to prepare a cyclized aliphatic phosphonitrilate⁹ and to prepare phosphonitrilate polymers.¹⁰

In the present investigation, phosphonitrilic chloride trimer (I) was treated with catechol and triethylamine

(1) Presented to the Symposium on Inorganic Polymers, University of Western Ontario, Sept., 1963.

(2) D. R. Dishon, *J. Am. Chem. Soc.*, **71**, 2251 (1949).

(3) M. Yokoyama and F. Yamada, *Kogakuin Daigaku Kenkyu Hokoku*, **7**, 62 (1959); *Chem. Abstr.*, **54**, 19458 (1960).

(4) C. Hamalainen and J. D. Guthrie, *Textile Res. J.*, **26**, 141 (1956).

(5) R. Ratz and M. Hess, *Ber.*, **84**, 889 (1951).

(6) B. W. Fitzsimmons and R. A. Shaw, *Chem. Ind. (London)*, 109 (1961).

(7) N. Yokoyama, *J. Chem. Soc. Japan*, **80**, 1192 (1959).

(8) R. F. W. Ratz and C. J. Grundmann, U. S. Patent 2,876,248 (1959).

(9) M. S. Chang and A. J. Matuszko, *Chem. Ind. (London)*, 410 (1962).

(10) C. A. Redfarn, U. S. Patent 2,866,773 (1958).