[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY, UNIVERSITY OF NEBRASKA COLLEGE OF MEDICINE]

Infrared Spectra of Chlorophyll Derivatives[1]

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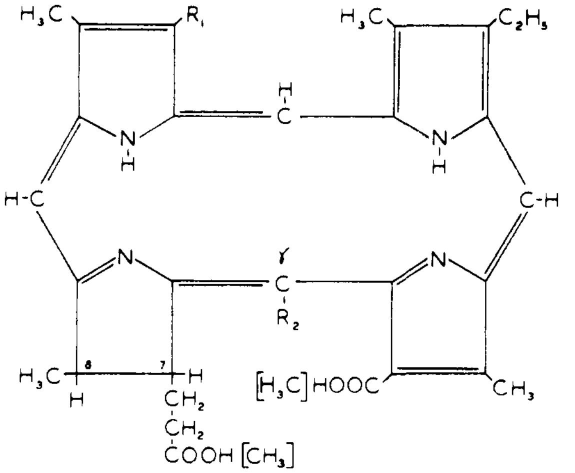
The infrared spectra of a number of chlorophyll derivatives in the 1500–3000 cm.$^{-1}$ region are presented, and important differences among chlorins, mesochlorins, porphyrins and 2-vinylporphyrins are noted. Where possible, assignments of the absorption bands to specific groupings have been made. The data indicate that steric hindrance of resonance is present in γ-substituted derivatives when compared with γ-unsubstituted compounds.

The cardiotonic activity of many of the chlorins and rhodins derived from chlorophylls *a* and *b* has been investigated in this department for some time. The preparation of these compounds for pharmacological investigations provided the opportunity to record their infrared spectra and to study the correlation between structure and spectrum. There have been several papers published on this subject,[2-4] but none has dealt with the dicarboxylic chlorins and mesochlorins. The purpose of this paper is to present the spectra (in the double bond stretching region) of a number of chlorophyll derivatives and several related prophyrins, and to explain some of the important differences between γ-substituted and γ-unsubstituted compounds, as well as differences among chlorins, mesochlorins porphyrins and 2-vinylporphyrins. Table I presents the structures of the chlorophyll derivatives under consideration. In order to avoid confusion in the carbonyl region from the 3-formyl group present in the *b* series, our measurements have been limited to members of the *a* series (see I for the numbering of the tetrapyrrole nucleus).

TABLE I

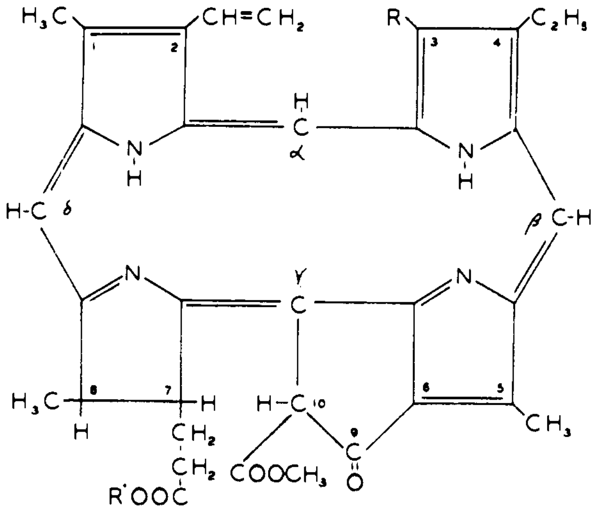


Compound	R₁	R₂
Rhodochlorin	CH═CH$_2$	H
Mesorhodochlorin	C$_2$H$_5$	H
Chlorin *e*$_4$	CH═CH$_2$	CH$_3$
Mesochlorin *e*$_4$	C$_2$H$_5$	CH$_3$
2-Vinylrhodoporphyrin	CH═CH$_2$	H[a]
Rhodoporphyrin	C$_2$H$_5$	H[a]
Purpurin 18*a*	CH═CH$_2$	COOH[b]

[a] Also dehydrogenation with double bond formation between C-7 and C-8. [b] Anhydride formed between carboxyls on C-γ and C-6.

Experimental

Preparation and Characterization of Compounds.—The preparation and preliminary purification of pheophorbide *a* (I, R = CH$_3$, R′ = H), and the chlorins and their corresponding porphyrins followed the standard methods of Willstätter[5] and Fischer.[6] Although not specifically recommended by Fischer, the reactions have been carried out, in most cases, in an atmosphere of nitrogen freed of oxygen and moisture by passage over a heated copper coil and thence through concentrated sulfuric acid and finally over pellets of potassium hydroxide. The dimethyl esters were prepared by means of diazomethane; methanol saturated at 0° with dry hydrogen chloride gas was used to prepare the propionic acid monomethyl esters[7]; mild alkaline hydrolysis of the corresponding dimethyl ester yielded the 6-monomethyl ester. The desired compound was purified by fractionation between ether and aqueous hydrochloric acid and, where possible, by extraction from ether solution with buffers of appropriate *p*H. This latter technique indicates the number of free carboxyls present.[8] Crystallization, chromatographic analysis[9] and visible absorption spectrophotometry (Beckman model DU) assured the homogeneity of each compound.



I

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(1) This work was supported in part by a Grant from the National Institutes of Health, National Heart Institute. Presented before the Organic Division at the 134th Meeting of the American Chemical Society, Chicago, Ill., September 10, 1958.

(2) J. E. Falk and J. B. Willis, *Austr. J. Sci. Research, Ser. A.*, **4**, 579 (1951).

(3) A. S. Holt and E. E. Jacobs, *Plant Physiol.*, **30**, 553 (1955).

(4) A. S. Holt, *Can. J. Biochem. and Physiol.*, **36**, 439 (1958).

(5) R. Willstätter and A. Stoll, "Investigations on Chlorophyll," translated from the German by F. M. Schertz and A. R. Merz, The Science Press Printing Co., Lancaster, Pa., 1928.

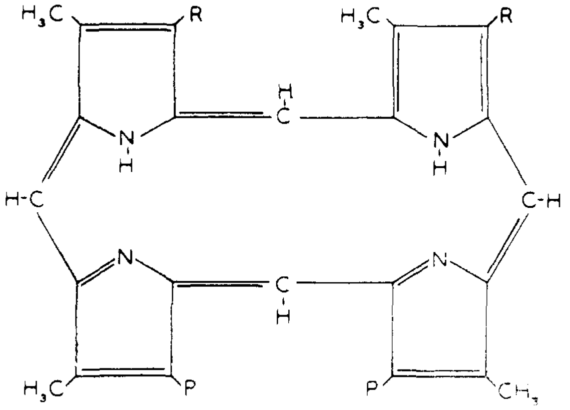
(6) H. Fischer and A. Stern, "Die Chemie des Pyrrols," Hälfte 2, Bd. II, Akademische Verlagsgesellschaft, Leipzig, 1940.

(7) M. Strell and E. Iscimenler, *Ann.*, **557**, 186 (1945).

(8) M. J. Hendrickson, unpublished observations.

(9) M. J. Hendrickson, R. R. Berueffy and A. R. McIntyre, *Anal. Chem.*, **29**, 1810 (1957).

Protoporphyrin IX dimethyl ester (II, R = $CH{=}CH_2$, P = $CH_2CH_2COOCH_3$) was prepared from sheep blood according to the method of Grinstein.[10] This was converted to mesoporphyrin IX dimethyl ester (II, R = C_2H_5, P = $CH_2CH_2COOCH_3$) by the method of Grinstein and Watson.[11]



II

Methyl indole-3-carboxylate, m.p. 147-148° (lit.[12] 147-148°) was prepared by treatment with diazomethane of indole-3-carboxylic acid, which had been obtained by permanganate oxidation of a commercial sample[13] of indole-3-carboxaldehyde according to Ellinger.[14]

Infrared Equipment and Methods.—All measurements were made with a Perkin–Elmer model 112 single beam spectrophotometer, equipped with sodium chloride optics and purged with dry nitrogen. The potassium bromide pellet technique was employed. About 0.5 mg. of the substance, intimately mixed with 500 mg. of potassium bromide, was adequate. When a duplicate spectrum was run in solution (chloroform solvent. sodium chloride cell. 0.1-mm. thick) as a check on the pellet method. there was good agreement between the two spectra. Although we have recorded our complete spectra from 700 to 5000 cm.$^{-1}$. this analysis will be restricted to the 1500–3000 cm.$^{-1}$ region.

Results and Discussion

In Table II are presented the chromatographic data obtained by the radial filter paper technique referred to earlier.[9] Figure 1 shows the infrared spectra.

Table II

Compound	R_f	Time, sec.	Solvent system[a]
Rhodochlorin dimethyl ester	0.93	3000	A
Mesorhodochlorin dimethyl ester	.96	1000	A
Chlorin e_4 dimethyl ester	.98	1500	A
Mesochlorin e_4 dimethyl ester	.97	1200	A
Rhodochlorin propionic acid monomethyl ester	.75	1000	A
Chlorin e_4 propionic acid monomethyl ester	.82	1000	A
Rhodochlorin-6-monomethyl ester	.61	1000	A
Purpurin 18*a* methyl ester	.81	3000	A
2-Vinylrhodoporphyrin dimethyl ester	.86	1500	B
Rhodoporphyrin dimethyl ester	.87	1500	B
Protoporphyrin IX dimethyl ester	80	3000	B
Mesoporphyrin IX dimethyl ester	.84	3000	B

[a] Solvent system: A. 10:20:70 (by volume) acetone–benzene–hexane; B, 20:20:60 (by volume) chloroform–benzene–hexane.

We have assigned the 1738 cm.$^{-1}$ band to the propionic acid methyl ester on the basis of the work of others on aliphatic acid esters[15] and also on our own studies on pyrrochlorin (prepared from rhodochlorin by decarboxylation at C-6), which has only this one ester residue.

On the possibility that one of the two strong peaks in the rhodochlorin spectra in the C=C stretching region (near 1610 and 1590 cm.$^{-1}$) was caused by the presence of the 2-vinyl group, mesorhodochlorin dimethyl ester was prepared and compared with rhodochlorin dimethyl ester. It can be seen that their spectra in this region are practically identical, thus indicating that the 2-vinyl group is not responsible for either. The only other work with which we are familiar that presents spectral data on a mesochlorin is that of Lautsch *et al.*,[16] Their spectrum of mesophyllochlorin methyl ester shows a peak at about 1613 cm.$^{-1}$, which we believe corresponds to the peak we find at about 1616 cm.$^{-1}$ in the chlorins and mesochlorins studied. It is also present in purpurin 18*a* and in all of the 7,8-dihydroprophyrins so far examined.[17] The bacteriochlorophyll derivatives (3,4,7,8-tetrahydroporphyrins) studied by Holt and Jacobs[3] possess a band near this location, although it appears to be somewhat weaker in intensity. This band is absent in the porphyrins. This last observation is the one we wish to emphasize since spectra of hexahydro- and octahydroporphyrins are not available for purposes of comparison. In order to make the unequivocal assignment to C=C or C=N stretching in the *reduced* porphyrin nucleus, such derivatives would have to be studied. Therefore, we wish to stress the absence of the band near 1610 cm.$^{-1}$ in the *true* porphyrins.

The other *strong* band in the C=C stretching region (near 1590 cm.$^{-1}$) is found only in "rhodo-type"[18] chlorins, and may be related to resonance interaction between the 6-carboxyl and the ring. which, as we shall point out. is decreased or absent in chlorins with a γ-substituent. A *weak* band appears here in the "rhodo-type" porphyrins.

The absorption in this region caused by the vinyl group therefore is not seen in the chlorins because of this strong interference from other moieties. It is uncovered in the porphyrins, however, and appears as a shoulder in 2-vinylrhodoporphyrin as compared with rhodoporphyrin; and is confirmed by comparing protoporphyrin with mesoporphyrin. The absorption due to CH and CH_2 out-of-plane deformation of the vinyl group near 1000 and 900 cm.$^{-1}$, respectively, is also masked in the chlorins. but we have observed it when comparing 2-vinylporphyrins with porphyrins.

The first compound we examined was rhodochlorin dimethyl ester, and although we believed that the band at 1700 cm.$^{-1}$ resulted from the

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(10) M. Grinstein, *J. Biol. Chem.*, **167**, 515 (1947).

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(12) C. Zatti and A. Ferratini, *Ber.*, **23**, 2296 (1890).

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(15) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958, pp. 179-181.

(16) W. Lautsch, W. Gehrmann, R. Pasedag and K. Prater, *Chem. Ber.*, **90**, 470 (1957).

(17) H. R. Wetherell, unpublished observations.

(18) "Rhodo-type" here refers to those compounds that possess a 6-COOH or $COOCH_3$ group and no substituent on the γ-position.

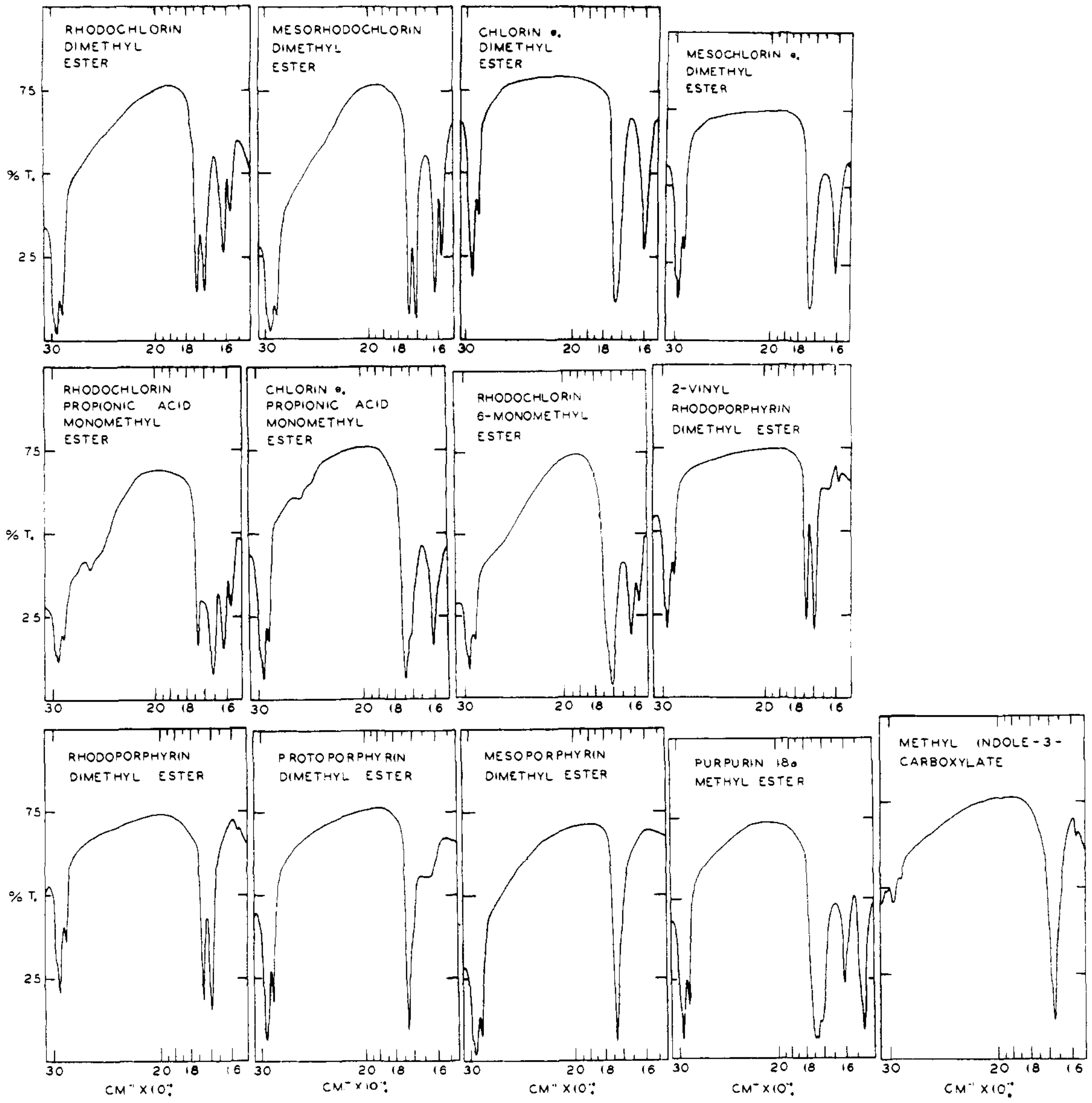

Fig. 1. - Infrared spectra of compounds: the abscissa is wave number and the ordinate is % transmission.

6-$COOCH_3$ group, the value seemed low. To establish this assignment we prepared the propionic acid monomethyl ester and the 6-monomethyl ester. The former retains the 1738 cm.$^{-1}$ ester band and the 6-carboxyl absorption appears at 1666 cm.$^{-1}$, a shift to be expected in the change from an ester to a free acid. In the spectrum of the 6-monomethyl ester only one broad band is seen. It results from the propionic acid and the 6 $COOCH_3$ bands at 1708 and 1700 cm.$^{-1}$, respectively, and these are too close for resolution. Methyl indole-3-carboxylate was prepared as a model compound, since its 3-carbomethoxy group might be expected to be somewhat analogous to the 6-carbomethoxy in the tetrapyrroles. It can be seen that the carbonyl absorption for this compound occurs at an even lower frequency.

When rhodochlorin dimethyl ester is compared with chlorin e_4 dimethyl ester, two striking differences are apparent. In the latter a single broad band is seen in the carbonyl region, and there is no band near 1590 cm.$^{-1}$. We suspected that the 6-$COOCH_3$ peak was shifted to such a high frequency that it was obscured by the propionic ester band. To check this possibility, chlorin e_4 propionic acid monomethyl ester was prepared in order to achieve maximum separation between the two carbonyl peaks. In this compound the 6-COOH now gives rise to a shoulder at about 1712 on the 1736 cm.$^{-1}$ band. We have interpreted this shift in location of the peak for the 6-substituent in going from rhodochlorin (with a H on C-γ) to chlorin e_4 (with a CH_3 on C-γ) as steric inhibition of resonance, the bulky γ-CH_3 group preventing the group on C-6 from becoming coplanar with the ring. The direction of the resultant shift is consistent with this explanation. The same relationships can be seen when mesochlorin e_4 is compared with mesorhodochlorin. We have observed similar shifts with compounds bearing other hindering groups on C-γ, *e.g.*, CH_2COOCH_3, $(CO)COOCH_3$ and $COOCH_3$.

The assignment of the band near 1590 cm.$^{-1}$ was based on its presence in "rhodo-type" chlorins and its absence in hindered chlorins, chlorins that have no COOH or COOCH$_3$ on C-6, and derivatives with an intact ring between C-γ and C-6, *i.e.*, phorbins and purpurin 18*a*. These marked differences in the infrared spectra between hindered and unhindered chlorins stand in distinct contrast to the similarity of their spectra in the visible region.

Bellamy[19] has cited a paper by Brown and Todd,[20] and has mentioned that the two carbonyl absorption bands associated with the anhydride structure are separated by only 34 cm.$^{-1}$ in the cyclic six-membered ring anhydride studied by these workers. This is in contrast to the bands in other types of anhydrides, which are about 60 cm.$^{-1}$ apart. We have studied one six-membered anhydride, purpurin 18*a* methyl ester, and find a difference of 31 cm.$^{-1}$, which is in satisfactory agreement with the work of these authors.

In each spectrum in Fig. 1 the bands near 3000 cm.$^{-1}$ result from C–H stretching. Although not shown, each compound had but one peak assignable to N–H stretching, and this was found near 3350 cm.$^{-1}$.

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[19] Reference 15, p. 128.

[20] B. R. Brown and A. R. Todd, *J. Chem. Soc.*, 1280 (1954).

[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY OF BOSTON UNIVERSITY AND THE RICE INSTITUTE]

Ionization Equilibria of Mono-*p*-methyl and Mono-*p*-trideuteriomethyl Trityl Chloride in Liquid Sulfur Dioxide[1]

BY NORMAN N. LICHTIN, EDWARD S. LEWIS, ELTON PRICE AND ROBERT R. JOHNSON

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The secondary isotope effect on the equilibrium of ionization of mono-*p*-methyl trityl chloride to the ion pair, $p\text{-CH}_3\text{C}_6\text{H}_5\text{C(Cl)(C}_6\text{H}_5)_2 \overset{K_1}{\rightleftharpoons} \overbrace{p\text{-CH}_3\text{C}_6\text{H}_5\text{C}^+(\text{C}_6\text{H}_5)_2\text{Cl}^-}$, due to replacing methyl protium with deuterium has been estimated at 0.16° by a method which employs conductance data at relatively low dilution (600 l. mole^{-1}). This method is 5 to 10 times as precise as comparison of equilibrium constants calculated from conductance data for higher dilutions. The value of $(K_1)_\text{H}/(K_1)_\text{D}$ per deuterium atom is estimated to be 1.003 ± 0.005.

Studies of reactions proceeding *via* transition states in which positive charge is delocalized from the reaction site into an aromatic ring have provided significant insight into the nature and scope of the secondary deuterium isotope effect. These have included investigation of the solvolysis in acetic acid and/or aqueous acetone of *m*- and *p*-methyl substituted α-phenylethyl chlorides, variously substituted with deuterium,[2] the solvolysis in aqueous ethanol and acetone[3] of several mono-*p*-alkylbenzhydryl chlorides substituted with deuterium at the α-carbon of the *p*-alkyl group and the nitration, mercuration and bromination of toluene substituted in the methyl group with deuterium or tritium.[4] In these reactions the replacement of α-hydrogen of a *p*-alkyl group by deuterium is associated with a kinetic isotope effect, k_H/k_D, which is quite small, ranging from a maximum of about 1.05 per deuterium atom[2] down to values indistinguishable from unity. This isotope effect appears to be dependent on the nature of the solvent.

This report presents the results of a study of the isotope effect on an equilibrium which is closely related to the assumed equilibria between reactants and transition states in the above cases. Earlier studies of the ion-forming equilibria of trityl chloride and many of its *o*-, *m*- and *p*-substituted derivatives together with data on the dissociation equilibria of many ionophores have provided a convenient means of evaluating substituent effects on the ionization equilibrium: $\text{Ar}_3\text{CCl} \overset{\text{SO}_2}{\rightleftharpoons} \overbrace{\text{Ar}_3\text{C}^+\text{Cl}^-}$.[5] The similarity of substituent effects in this system to those observed in reactions like the solvolyses and electrophilic substitution reactions cited above has recently been elegantly demonstrated.[6] Studies of these equilibria provide a measure of substituent effects where full carbonium ion character is unequivocally developed.[7]

Equilibrium Constants.—Initially, it was hoped that thermodynamic equilibrium constants, K_exp, evaluated from conductance data collected by our usual methods[5] by means of Shedlovsky's procedure[8] could be determined with sufficient precision to provide a useful measure of the secondary deuterium isotope effect. In order to maximize the accuracy of the comparison, an unusually large number of conductance runs were carried out;

[1] Paper VII in the series "Ionization and Dissociation Equilibria in Liquid Sulfur Dioxide." Paper IV in the series "Isotope Effects in Carbonium Ion Reactions."

[2] (a) E. S. Lewis and G. M. Coppinger, THIS JOURNAL, **76**, 4495 (1954); (b) E. S. Lewis, R. R. Johnson and G. M. Coppinger, *ibid.*, **81**, 3140 (1959).

[3] V. J. Shiner, Jr., and C. J. Verbanic, *ibid.*, **79**, 373 (1957).

[4] C. G. Swain, T. E. C. Knee and A. J. Kresge, *ibid.*, **79**, 505 (1957).

[5] *Cf.* N. N. Lichtin and M. J. Vignale, *ibid.*, **79**, 579 (1957), and earlier papers.

[6] Y. Okamoto and H. C. Brown, *ibid.*, **80**, 4986 (1958).

[7] The absorption spectrum of the ionophore triphenylcarbonium perchlorate dissolved in liquid sulfur dioxide has been shown by P. D. Bartlett and R. E. Weston, Jr., to be identical with the spectrum of triphenylcarbinol dissolved in concentrated sulfuric acid (*cf.* ONR Technical Report No. 6 under Project No. NR-056-095, Contract No. N5 ori-76, Task xx, April 10, 1952).

[8] T. Shedlovsky, *J. Franklin Inst.*, **225**, 739 (1938).