

## NOTES

## The Isolation of Vitamin K as a Choleic Acid

BY H. J. ALMQUIST AND A. A. KLOSE

We wish to report the preparation of vitamin K choleic acid, a molecular compound of the vitamin with deoxycholic acid. This compound may prove useful in isolating the pure vitamin, especially since the vitamin appears inert to reagents that attack specific groups [*J. Biol. Chem.*, **125**, 681 (1938)].

Concentrates prepared by methods already noted [THIS JOURNAL, **61**, 532 (1939)] were dissolved in warm ethanol and an amount of deoxycholic acid equal to five times the weight of the concentrate was added. The solution was evaporated to dryness using gentle heat and a stream of inert gas. The solid was then dissolved in approximately 2 cc. of methanol per 100 mg., and 0.5 cc. of water was added. After thorough mixing and cooling at 0° for several hours, the precipitate was filtered off and dried. In some cases the product was recrystallized by the same procedure. When working with cruder concentrates such as those which we obtain after molecular distillation, it was found preferable to add water to the methanol solution to the point of distinct turbidity only, and to reject the first crop of crystals,

TABLE I  
RESULTS ON VITAMIN K CHOLEIC ACIDS AND RELATED PREPARATIONS

Preparation	M. p., °C.	Level fed per kg. of diet, mg.	Average blood clotting time, minutes	Number of chicks
F 1	195-196	100	>30	5
F 2	182-184	100	4.3	7
D 1	194-195	125	>17	4
D 2	185-186	125	3.1	4
M 1	182-184	75	4.0	9
M 1, xylene extract of 75 mg.	(oil)	≈75	4.5	9
M 1, solids in mother liquor	170-172	200	>30	8
H 1	186-187	....	...	...
H 1, molecular distillate	(oil)	7.5	4.3	8
H 1, distillation residue		100	>30	5
E 1 (from purified concentrate)	186-187	....	...	...
O 1 (from purified concentrate)	186-187	75	3.7	5
Reference standard solution <sup>a</sup>	(oil)	16 cc.	4.0	20
Purified concentrate	(oil)	5	7.4	7

<sup>a</sup> Standard solution representing 1 g. of dried alfalfa per cc.

which was usually inactive. Fractional crystallization was continued by concentrating the solution or by adding more water to it. In Table I are given representative data on various fractions obtained and their vitamin K activities.

As shown in the case of preparations F and D which were made from less pure concentrates, first an inactive fraction with a melting point at approximately 195° has been obtained. A second fraction melting at approximately 185° has proved quite active. Third and fourth fractions and the residual solids in the mother liquors, all of much lower melting points than the second fraction, invariably have shown no activity. The active fraction has been successfully decomposed by extraction with xylene and by molecular distillation.

From the purest concentrates yet prepared, we have repeatedly obtained a vitamin K choleic acid with a melting point of 186-187°. No other fraction, except deoxycholic acid itself, could be isolated. Activity of these preparations has also been tested by oral administration to deficient chicks. Restoration of normal clotting time occurred within twenty-four hours, indicating high activity. An ether extract of a sample of vitamin K choleic acid was tested and found active in the same way.

From preliminary titrations of the choleic acid, we estimate that it contains about 10% vitamin K. Thus some of the most active preparations fed at a level of 75 mg. per kilogram have furnished 7.5 mg. of the vitamin, with resulting low blood clotting times which approximate the blood clotting times from feeding the purified concentrate.

In interpreting these values, it must be recalled that the assay method we now use [*Biochem. J.*, **32**, 1897 (1938)] is more rigorous than our former methods, inasmuch as chicks are depleted of vitamin K for one week before use on assay. A comparison of blood clotting time results must take into account the assay methods and the standards employed.

The vitamin K choleic acid is a crystalline substance with a slight yellow color. When liberated from the choleic acid and from traces of solvent and deoxycholic acid, the vitamin has reap-

peared as a viscous, slightly pigmented oil. It bears no resemblance in physical properties to a crystalline product with a melting point of 69° claimed to be pure vitamin K by Doisy and co-workers [*Science*, **88**, 243 (1938)].

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### Relation between Compressibility and Solubility of Metals in Acids

BY SOFRONIO BALCE

If we apply to metals the compressibility formula for compounds as given in an earlier paper,<sup>1</sup> the equation becomes

$$B_{\text{calcd.}} = \frac{V_a}{5.6 \times 10^{12} Z}$$

where  $B_{\text{calcd.}}$  is the calculated compressibility,  $5.6 \times 10^{12}$  is a universal constant relating physical

properties of substances to shrinkage in volume per unit volume incident to compound formation,  $Z$  is the valence of the metal elements, and  $V_a$  the atomic volume.

This calculated compressibility, however, does not check with observed values. But when the  $B_{\text{calcd.}}$  calculated compressibility is less than the  $B_{\text{obsd.}}$  observed value, the metal may be said to be comparatively soft and is subject to solvent action by non-oxidizing acids; and when the calculated compressibility is greater than the observed, the metal does not displace the hydrogen.

As may be noted in the accompanying table, the order in which the metals occur in the electro-motive series can be approximated by dividing the deviation,  $\Delta B = B_{\text{calcd.}} - B_{\text{obsd.}}$  by  $B_{\text{calcd.}}$ . The units of compressibility in the table are changed to cc./atmosphere.

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TABLE I

SHOWING THE DIFFERENCES BETWEEN METALS THAT DISPLACE HYDROGEN FROM ACIDS AND THOSE THAT DO NOT

Metal	Z	Compressibility <sup>a</sup> in cc./atm.		$B_{\text{obsd.}} - B_{\text{calcd.}}$
		$B_{\text{calcd.}}$	$B_{\text{obsd.}}$	
Cs	1	$12.9 \times 10^{-6}$	$62 \times 10^{-6}$	3.81
Li	1	2.35	9.1	2.87
Rb	1	10.5	40.5	2.86
K	1	8.54	32.1	2.76
Na	1	4.3	15.8	2.67
Sr	2	3.05	8.1 <sup>b</sup>	1.65
Ca	2	2.29	5.8	1.53
Mg	2	1.21	2.9	1.4
Al	3	0.61	1.34	1.2
Zn	2	.86	1.45	0.68
Cr	3	.45	0.73 <sup>c</sup>	.64
Fe	3	.43	.654	.52
Cd	2	1.17	1.72	.47
Co	3	0.405	0.557	.375
Ni	3	.40	.542	.364
Sn	2	1.47	1.9	.29
Pb	2	1.65	2	.23
Cu	1	1.29	0.756	-.41
Ag	1	1.85	1.02	-.45
Pt	2	0.82	0.328	-.60
Au	1	1.845	.552	-.66
Ir	2	0.778	.244	-.89

<sup>a</sup> The figures on compressibility are from the "International Critical Tables."

<sup>b</sup> P. W. Bridgman, *Proc. Am. Acad.*, **70**, 285-317 (1935).

<sup>c</sup> T. W. Richards, "The Compressibilities of the Elements and Their Periodic Relations," Carnegie Institution of Washington, 1907.

(1) S. Balce, *Philipp. J. Sci.*, **60**, 251-254 (1936) [*Chem. Zentr.*, **108**, II, 2113 (1937); *British C. A.*, A, I, 176 (1937); *C. A.*, **81**, 2881 (1937)].

### Note on the Solubility of Strontium Chromate

BY T. W. DAVIS AND J. E. RICCI

In connection with an attempted study of the solubility of strontium chromate in dioxane-water mixtures as solvents, which had to be abandoned because of the extreme slowness with which equilibrium is approached, some observations were made on the solubility of this salt in water.

The figure uniformly given for the solubility of strontium chromate at room temperature is the determination of Fresenius,<sup>1</sup> 0.12 g. in 100 g. of solution at 15°, which was a confirmation of work by Meschtschersky.<sup>2</sup>

The only values for other temperatures are those of Reichard<sup>3</sup>: namely, 0.465% at 10°, 1.000% at 20°, 2.417% at 50° and 3.000% at 100°. The last figure for 100°, is the one quoted in the "Handbook of Chemistry and Physics,"<sup>4</sup> in its current editions. These incredible figures are evidently the basis for the Noyes procedure for the qualitative analysis of the alkaline earths, in which one is cautioned against much washing of the strontium chromate precipitate which is then redissolved by passing hot water through the filter paper.

(1) Fresenius, *Z. anal. Chem.*, **29**, 418 (1890).

(2) Meschtschersky, *ibid.*, **21**, 399 (1882).

(3) Reichard, *Chem. Zig.*, **27**, 877 (1903).

(4) Chemical Rubber Publishing Co., Cleveland, Ohio.

Reichard's experimental procedure is not clear from his description, but something is unquestionably wrong with his determinations. Neither Meschtschersky nor Fresenius mentions the time allowed for attainment of equilibrium.

We have noted first of all that equilibrium is approached extremely slowly; we are in fact not prepared to give figures indicating true solubility in every case. As shown by Kohlrausch<sup>5</sup> for anhydrous calcium chromate, the time required for this type of salt may be a matter of years. The solubility of anhydrous calcium chromate, which he was determining, was still increasing after fifteen months. Our observations nevertheless are sufficient to show beyond doubt that the solubility of strontium chromate decreases markedly with rising temperature, which was quite unexpected, considering that the opposite had long been assumed in analytical procedure. The success of the Noyes procedure for Group IV metals must be attributed to the much greater rate of solution of the precipitate in hot as compared to cold water.

The strontium chromate used was prepared from c. p. strontium chloride and c. p. ammonium chromate; it was recrystallized from large volumes of water several times by redissolving in hydrochloric acid and reprecipitating with ammonium hydroxide. The product was finally digested in hot water for long periods, to approach a granular condition. Analysis of the solid gave 99.5% strontium chromate by iodometric titration with standard thiosulfate. This same analytical method was used in the solubility determinations themselves. The procedure for the latter was to stir an excess of solid with 200–250 cc. of water in glass-stoppered Pyrex bottles, in electrically controlled thermostats, at 15, 25 and 75°. For the 100° determination, excess solid was boiled with 1.5 liters of water in a 2-liter Pyrex flask fitted with a small reflux condenser.

The results obtained are shown in Table I. The figures for 15 and 25° probably do not represent equilibrium, although the value 0.91 g./l. at 25° is probably very close to a true solubility. The attainment of equilibrium at 15° must of course be much slower, so that it is likely that the 15° figure would have continued to rise to a value higher than that found at 25°. The 75° figure probably represents equilibrium. Here the rate of reaching equilibrium must be quite high, and

(5) Kohlrausch, *Z. physik. Chem.*, **44**, 233 (1903).

the values from undersaturation and supersaturation agree very closely. The 100° value is probably correct as far as its order of magnitude is concerned, but cannot be very precise. In this determination the continued boiling of the mixture caused an increase in the  $\text{CrO}_4^{2-}$  concentration of the solution following, after the first day or two, a linear course which was not that expected for a change approaching equilibrium in the usual logarithmic fashion, but one in accordance with the assumption that either an interchange with the glass was taking place, with a consequent increase in the  $\text{CrO}_4^{2-}$  concentration, or a progressive hydrolysis of the solid, with the same effect. To correct for this change, on the reasonable assumption that solubility equilibrium must be reached rather quickly at this temperature, duplicate experiments were run, using very different amounts of solid, in which the solution was sampled every day or two, for periods up to nine days; the values so obtained, when a rough linearity had been established, were extrapolated back to zero time, to give the saturation value without the effect of hydrolysis or exchange with glass. The most probable values of the solubility, omitting the 15° figures, are listed in Table II.

It may be added, in conclusion, that the solid phase was analyzed and found to be anhydrous,

TABLE I

Temp., °C.	Expt.	Time, days	Soly., G./l. soln.	
15	1	20	0.741	
		25	.788	
	2	20	.779	
		25	.879	
	25	1	3	.833
			27	.865
31			.864	
2		18	.909	
		22	.912	
		24	.898	
75	From undersaturation	10	0.6143	
	From supersaturation	10	.6150	
100 (extrapolated to zero time)	1		.43	
	2		.42	

<sup>a</sup> Using residue from 100° determination.

TABLE II

Temp., °C.	Solubility
25	0.91 g./l. soln.
75	.615 g./1000 g. solution
100	.43 g./1000 g. solution

both at 25 and at 100°: at 25°, 97–98.0% strontium chromate by titration; at 100°, 96.7%.

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### The Identification of Polynitro Aromatic Compounds as Addition Compounds with Naphthalene<sup>1</sup>

BY O. C. DERMER AND R. B. SMITH

Although polynitro aromatic compounds are not rare in industry and are common derivatives for identifying other aromatic compounds, there is no systematic procedure for their own identification. As a class they can be detected by color reactions,<sup>2</sup> but their derivatives generally owe their existence to the reactivity of some other functional group in the molecule. If no such group is present, recourse must be had to either (a) reduction, which is difficult to control and frequently yields polyamines requiring acylation to make them suitable derivatives, or (b) further nitration, which may be impossible.

In view of the well-known excellence of picric acid and other polynitro aromatic compounds as reagents for identifying condensed-ring aromatic compounds, the reverse procedure, suggested by Mulliken<sup>3</sup> and Clarke<sup>4</sup> and more explicitly by Reichstein<sup>5</sup> and Coghill and Sturtevant,<sup>6</sup> seemed worth systematic extension. The attractiveness of the method was increased by a literature search, which showed that 39 polynitro compounds of the 47 that had been studied form complexes with naphthalene. We retained naphthalene as the reagent because of this prior use and because it is universally available and easy to purify.

We chose for study polynitro compounds which either are commercially available and therefore

commonly encountered, or are themselves derivatives used in qualitative organic analysis. In each case the polynitro compound-naphthalene system was investigated by an application of cooling-curve technique to the method used by Baril and Hauber<sup>7</sup> for hydrocarbon picrates. If the curve for the equimolecular mixture indicated compound formation, the stability of the supposed compound toward recrystallization was tested. If recrystallization caused decomposition, we usually considered it necessary to construct the whole melting point-composition diagram, since a plateau on the cooling curve might represent merely the crystallization of a eutectic mixture at the fifty mole per cent. point.

Of the seventeen new derivatives thus found and described in Table I, all but four can be recrystallized without decomposition. Even these four can be used in qualitative analysis; but inasmuch as the molecular weight must be known before an equimolecular mixture can be made, this method without recrystallization is merely confirmatory

TABLE I  
NEW DERIVATIVES OF POLY-NITRO COMPOUNDS

M. p., °C. (corr.)	Polynitro compound	M. p. of C <sub>10</sub> H <sub>8</sub> deriv., °C. (corr.)
62	Isoamyl 3,5-dinitrobenzoate <sup>b</sup>	46–47
63	2,6-Dinitrophenol <sup>c</sup>	58–58.5
68	2,4,6-Trinitroanisole	69–70 <sup>f</sup>
79	2,4,6-Trinitrophenetole	39
86	2,4-Dinitrophenetole	41 <sup>d</sup>
86.5	3,5-Dinitro- <i>o</i> -cresol	94
88	1-Iodo-2,4-dinitrobenzene <sup>c</sup>	66–67
89	2,4-Dinitroanisole	50
93	Ethyl 3,5-dinitrobenzoate	75 <sup>f</sup>
98	Ethyl 3,5-dinitrosalicylate	78 <sup>d,f</sup>
106	2,4-Dinitro-6-cyclohexylphenol	73–74
106	3,5-Dinitroanisole	69 <sup>d</sup>
117	3-Chloro-2,4,6-trinitrophenol	127
119	2,4,6-Trinitrobenzaldehyde <sup>c</sup>	136.5
123	3,5-Dinitroguaiacol	94
147	2,4-Dinitroresorcinol	165
171	2,4,2',4'-Tetranitrobenzyl <sup>g</sup>	136 <sup>d,f,h</sup>

<sup>a</sup> Capillary melting points are given unless otherwise stated. The melting points from cooling curves are generally about 2° lower. <sup>b</sup> Ref. 5. <sup>c</sup> Eastman Kodak Co. product. <sup>d</sup> Value obtained from the cooling curve only; compound could not be recrystallized without decomposition. <sup>e</sup> Körner, *Gazz. chim. ital.*, **4**, 323 (1874). <sup>f</sup> Result verified by melting point-composition diagram. <sup>g</sup> Braun and Rawicz, *Ber.*, **49**, 802 (1916). <sup>h</sup> Compound may melt incongruently; the temperature-composition curve was not determined accurately enough to decide the question. <sup>i</sup> Asahina and Shinomiya, *J. Chem. Soc. Japan*, **59**, 341 (1938), reported 54° for this m. p.

(7) Baril and Hauber, *THIS JOURNAL*, **53**, 1087 (1931).

(1) This is an abstract of a thesis submitted by R. B. Smith in partial fulfillment of the requirements for the degree of Master of Science at the Oklahoma Agricultural and Mechanical College in 1938. Nearly all this material was presented at the Milwaukee meeting of the American Chemical Society, September, 1938.

(2) Houben, "Die Methoden der organischen Chemie," G. Thieme, Leipzig, 3rd edition, Vol. IV, 1924, pp. 190–192.

(3) Mulliken, "A Method for the Identification of Pure Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., Vol. II, 1916, Suborder II, various pages.

(4) Clarke, "A Handbook of Organic Analysis," Edward Arnold and Company, London, 1928, pp. 240–241.

(5) Reichstein, *Helv. Chim. Acta*, **9**, 799 (1926); Sutter, *ibid.*, **21**, 1266 (1938).

(6) Coghill and Sturtevant, "An Introduction to the Preparation and Identification of Organic Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1938, p. 202.

and requires previous supporting evidence. Twenty-nine polynitro compounds yielded no 1:1 complexes with naphthalene: 2,4-dinitrobenzaldehyde, 2,4-dinitrodiphenyl ether, 2,4-dinitrodiethylaniline, 2,4-dinitromesitylene, 2,6-nitrohydroquinone-4-acetate, 2,4-dinitrobenzyl acetate, 1-*t*-butyl-3,5-dimethyl-2,4,6-trinitrobenzene, 2,4-dinitrobenzyl alcohol, 2,4-dinitroacetanilide, 2,2'-dinitrobiphenyl, 4,5-dinitroveratrole, 2,6-dinitrohydroquinone, 2,4-dinitro-1-naphthol, 2,3,4,6-tetranitrophenol, 3,4,5-trinitroveratrole, 2,4-dinitro-1-naphthol-7-sulfonic acid, 2,4-dinitrodiphenylamine, 2,4,2',4'-tetranitrobiphenyl (eutectic at about 85 mole % naphthalene), picramic acid, 2,4,2',4'-tetranitrodiphenylmethane (eutectic at about 90 mole % naphthalene), 1,8-dinitronaphthalene, 2,4-dinitrophenylacetic acid, 2,4-dinitrophenylhydrazine, 3,3'-dinitrobiphenyl, 3,5-dinitrobenzoic acid, 1,3,8-trinitronaphthalene, 2,4,6-trinitrobenzoic acid, 4,4'-dinitrobiphenyl.

Although the method of identification outlined in this paper is not general in application, it has compensating desirable features. It is simple, rapid, and inexpensive, and it gives derivatives from which the only possible impurities, the original reagents, usually may be removed by recrystallization.

The attempt to deduce rules correlating structure of a polynitro compound with its ability to yield a stable complex is beset with difficulties. Although it is clear that polynitrophenols usually give stable derivatives, which in many cases even melt higher than either of their constituents, there are several exceptions. Phenolic ethers mostly resemble the phenols in behavior, but the complexes are much less stable. At the other extreme are polynitro amines, which rarely yield compounds with naphthalene.<sup>8</sup> Our results on 2,4-dinitromesitylene and 1-*t*-butyl-3,5-dimethyl-2,4,6-trinitrobenzene confirm earlier reports<sup>9</sup> of the hindering effect of methyl groups situated between nitro groups on the ability of the latter to cause compound formation.

As might have been predicted for polynuclear compounds, at least two nitro groups must be present on each ring if addition compounds are to be formed<sup>10</sup>; but their relative positions on a ring

are also of consequence. In particular, nitro groups ortho to each other both seem to diminish in ability to promote reaction. This is obvious in the cases of *o*-dinitrobenzene and 3,4-dinitrotoluene,<sup>11</sup> and may explain the failure of 2,3,4,6-tetranitrophenol, 2,3,4,6-tetranitroaniline,<sup>12</sup> and the polynitroveratroles to yield complexes; but the generalization fails to account for the activity of 2,3,4-trinitrotoluene, 4,5,6-trinitro-*o*-cresol, and several polynitrochlorobenzenes.<sup>13</sup>

The preponderance of high-melting polynitro compounds among those which formed no addition products suggests that the dominant factor is not structure, but melting point, of the polynitro compound; and of course high melting point connotes lowered mutual solubility of naphthalene and the polynitro compound.

### Experimental

The naphthalene used was a commercially resublimed sample which we recrystallized from alcohol. The other principal reagents were prepared or otherwise obtained as shown in Table I.

The apparatus employed for determining cooling curves was patterned after that of Washburn.<sup>14</sup> To avoid the need of a specially constructed Dewar flask, three test-tubes of different sizes were fitted together concentrically by cork stoppers. The thermometer, which served also as a stirrer, was supported loosely in the small central tube by another cork stopper.

Ten millimoles each of naphthalene and the polynitro aromatic compound were fused together and transferred to the central tube of the apparatus. The whole apparatus was heated until the material melted, and then allowed to cool slowly, supercooling of the melt being minimized by stirring and seeding. It was thus possible to obtain reproducible cooling curves, on which plateaus parallel to the time axis were considered evidence of compound formation. The thermometer was calibrated in the apparatus by using compounds of known purity and melting points: diphenylamine, naphthalene, and benzoic acid.

The compounds indicated to exist were recrystallized, usually from the minimum amount of alcohol, and then examined under the microscope for homogeneity. If dissociation had occurred, we used Rheinboldt's "thaw-point" technique<sup>15</sup> to learn the form of the melting point-composition curve, since this is a rapid semi-micro method

*ibid.*, 1108 (1936)] have shown that mononitro compounds containing electron attracting groups in both meta positions can yield addition compounds. Since Hammick and Hellicar<sup>10</sup> claim this kind of reinforcing influence for chlorine, we have examined the behavior of 3,5-dichloronitrobenzene and found it to give no 1:1 compound with naphthalene. Evidently the electron attracting power of chlorine is not so great as that of the groups employed by Bennett.

(11) Kremann and Rodinis, *Monatsh.*, **27**, 125 (1906).

(12) Davis and James, *Aberystwith Studies*, **4**, 213 (1922).

(13) Beilstein, "Handbuch der organischen Chemie," Verlag von J. Springer, Berlin, 4th edition.

(14) Washburn, *Ind. Eng. Chem.*, **16**, 275 (1924).

(15) Rheinboldt, in Houben, "Die Methoden der organischen Chemie," G. Thieme, Leipzig, 3rd ed., Vol. I, 1925, p. 1169.

(8) Compare Buehler, Hisey and Wood, *THIS JOURNAL*, **52**, 1939 (1930).

(9) (a) Efremov and Tikhomirova, *Ann. inst. anal. phys. chim.*, **4**, 85 (1928); (b) Hammick and Hellicar, *J. Chem. Soc.*, 761 (1938).

(10) Cf. Hertel and Kurth, *Ber.*, **61**, 1650 (1928). No exceptions involving polynitro compounds are known, but Bennett and co-workers [Bennett and Willis, *ibid.*, 266 (1929); Bennett and Wain,

and yet accurate enough to prove whether a molecular compound exists.

A condensed procedure for this method of identification is as follows. Fuse together as nearly as possible equimolecular amounts of naphthalene and unknown, cool, recrystallize the melt from a little alcohol, and determine the melting point of the crystals.

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## The Identification of Olefins as Dithiocyanates<sup>1</sup>

BY O. C. DERMER AND GEORGE A. DYSINGER

The only general method of converting olefins into solid derivatives suitable for identifying them is the addition of nitrosyl chloride or oxides of nitrogen, and even the derivatives so obtained often must be changed into the more stable nitrolamines. A recent suggestion<sup>2</sup> that olefins be identified as addition products with osmium tetroxide can hardly be expected to improve the situation. Since Bruson and Calvert<sup>3</sup> demonstrated the usefulness of thiocyanogen for identifying isoprene and 2,3-dimethylbutadiene-1,3, and since thiocyanogen is known to add to several olefins, including even ethylene, with the formation of crystalline solids,<sup>4</sup> we thought it desirable to test the method further, particularly with aliphatic and alicyclic olefins.

Thiocyanogen was obtained either from sodium thiocyanate and cupric sulfate<sup>5</sup> or by the action of bromine on plumbous thiocyanate.<sup>4a</sup> Its addition to a number of common olefins produced two solid dithiocyanates already known and only two new solids: ethylene dithiocyanate, m. p. 90.0–90.5°; styrene dithiocyanate,<sup>3,4b,4c</sup> m. p. 102.5–103.0°; cyclohexene dithiocyanate, m. p. 58.0–58.5°; and 3-methylcyclohexene-1 dithiocyanate, m. p. 69.5–70.0°. Most of the compounds tested yielded only oily dithiocyanates which were not further investigated; this group included propylene,<sup>6</sup> butene-1, butene-2,<sup>7</sup> 2-methylpropene-1,<sup>6</sup> pentene-2,<sup>7</sup> 1-methylbutene-2, caprylene, diiso-

(1) This is an abstract of a thesis submitted by Mr. Dysinger in partial fulfillment of the requirements for the degree of Master of Science at the Oklahoma Agricultural and Mechanical College in 1938.

(2) Criegee, *Angew. Chem.*, **51**, 519 (1938).

(3) Bruson and Calvert, *This Journal*, **50**, 1735 (1928).

(4) (a) Söderbäck, *Ann.*, **419**, 217 (1919); (b) Kaufmann and Liepe, *Ber. pharm. Ges.*, **33**, 139 (1923); (c) Kaufmann and Oehring, *Ber.*, **59**, 187 (1926); (d) Müller and Freytag, *J. prakt. Chem.*, **146**, 58 (1936).

(5) Kaufmann and Kùchler, *Ber.*, **67**, 944 (1934).

(6) Hagelberg, *ibid.*, **23**, 1086 (1890).

(7) Likhosherstov and Butrimov, *Acta Univ. Voronegiensis*, **8**, No. 4, 86 (1935).

butene, *d*-limonene, pinene, diallyl, allyl alcohol,<sup>4b</sup> and allyl bromide. All the derivatives, like thiocyanogen itself, have a disagreeable odor and an irritant action on the skin. It is likely that selenocyanogen, (SeCN)<sub>2</sub>, would give derivatives with higher melting points,<sup>6</sup> but the difficulty of obtaining metallic selenocyanates and the unpleasant characteristics of the reagent and derivatives make the usefulness of the method in qualitative organic analysis very doubtful.

## Experimental

The olefins were either purchased from supply houses or synthesized by usual procedures. In Method A, about 10 g. of the olefin was added to a well-shaken suspension of 60 g. of sodium thiocyanate and 60 g. of anhydrous cupric sulfate in 150 ml. of glacial acetic acid. This mixture was allowed to stand overnight in the ice-box and was then filtered and diluted with much water. The product was removed by another filtration or by extraction with benzene, and if solid was recrystallized from dilute alcohol.

In Method B, the olefin was added to a suspension of 20 g. of plumbous thiocyanate in 100 ml. of thiophene-free benzene to which had been added 8 g. of bromine. The mixture was kept in an ice-bath in direct sunlight for about an hour or until its brown color disappeared. It was then filtered, the benzene evaporated, and the product purified as in Method A.

The methods seemed about equally useful, some olefins being treated by one, some by the other, and some by both. Cyclohexene dithiocyanate was prepared by Method A; it is a white crystalline compound, m. p. 58.0–58.5°, which was analyzed for sulfur.

*Anal.* Calcd. for C<sub>6</sub>H<sub>10</sub>N<sub>2</sub>S<sub>2</sub>: S, 32.25. Found: S, 32.36.

3-Methylcyclohexene-1 dithiocyanate, likewise made by Method A, forms white crystals melting at 69.5–70.0°.

*Anal.* Calcd. for C<sub>9</sub>H<sub>12</sub>N<sub>2</sub>S<sub>2</sub>: N, 13.21. Found: N, 12.96.

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## Sodium Ethylene Carbide

BY O. C. DERMER AND CLARENCE LATHROP

In 1927 Walker<sup>1</sup> reported that when ethylene is passed over metallic sodium at 150°, a little of the ethylene is polymerized to a colorless oil, and the sodium turns white. To quote: "A white brittle solid was formed—sodium ethylene carbide—and the condensation of colorless oil decreases. Finally all the sodium is converted to this new brittle material which has no reaction with ethylene and the polymerization stops." These re-

(1) Walker, *J. Phys. Chem.*, **31**, 982–984 (1927).

sults have been cited among ethylene reactions by Egloff<sup>2</sup> but apparently have never had experimental confirmation.

As such a reaction would be of great interest in both synthetic work and theoretical discussions, we have attempted to produce it, but our efforts have been uniformly unsuccessful. Impure ethylene does yield a white solid when passed over or bubbled through molten sodium at 150°, but if the ethylene be purified by bubbling it through one or two test-tubes of the metal at this temperature, it does not react at all with more sodium. Such tarnishing of the metal as does then occur is due to air and moisture adsorbed on glass surfaces in the apparatus, for it can be diminished greatly by flaming the container tube and connections before use. The white solid that was formed in the scrubber tubes by the impure ethylene did not have the reactivity which would be expected of an organometallic compound of sodium. We were likewise unable to find any evidence of polymerization of the ethylene. Indeed, according to Walker's own data, little or no ethylene was consumed by polymerization or reaction with sodium, and the purity of the gas actually increased during the reaction.

(2) Egloff, "The Reactions of Pure Hydrocarbons," Reinhold Publishing Corp., New York, N. Y., 1937, p. 288.

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### Refraction Data on Liquid C<sub>3</sub>-Hydrocarbons

BY ARISTID V. GROSSE AND CARL B. LINN

One of us<sup>1</sup> recently has described the adaptation of the Abbé refractometer to low temperature measurements.

We have now completed measurements of the refractive indices for the D-line, at low temperatures, of *all known* C<sub>3</sub>-hydrocarbons with the exception of cyclopropene.<sup>2</sup> (Very little is known about this hydrocarbon and none of its physical properties have ever been measured.) In order to obtain the molecular refractions we have determined, with Mr. R. C. Wackher, the still unknown densities of cyclopropane and allene.

In the following table the  $n_D$  and  $d_4^{25}$  (corrected to vacuum) of *propane*, *propene*, *cyclopropane*, *propyne* (allylene) and *propadiene* (allene) are recorded for the temperature range of -20 to -80° and also at their respective normal boiling points.

(1) A. V. Grosse, *THIS JOURNAL*, **59**, 2739 (1937).

(2) N. J. Demjanow and M. Dojarenko, *Ber.*, **56**, 2200 (1923).

The C<sub>3</sub>-hydrocarbons show wide differences in their indices. These may be used advantageously for *analytical purposes* since binary mixtures of these hydrocarbons show with sufficient accuracy a straight line relationship. Particularly interesting cases are the propane-propene mixtures, obtained in catalytic dehydrogenation, or propyne-propadiene mixtures from isomerization studies.

The molecular refractions of these hydrocarbons are of interest since they are the lowest members of the series so far studied in the liquid state. The *Lorenz-Lorentz refractions* were calculated from the data of Table I for different temperatures and the values extrapolated to +20° are compared with values computed from Eisenlohr's atomic refractions in Table II. Molecular volumes are included for comparisons.

The experimental molecular refractions (extrapolated to +20°) are in as good agreement with the calculated values as can be expected in view of their large temperature coefficients. These variations with temperature are much greater than for higher molecular weight compounds, measured around room temperatures; however, from the standpoint of the theory of corresponding states this is at least partly to be expected, since the absolute position and range of our measurements cover about 20% of the total distance from absolute zero to the normal boiling point.

The only real deviation is observable in the case of cyclopropane. The difference, equalling 0.4 unit, evidently is due to the exaltation of the cyclopropane ring. This exaltation value is slightly less than the one obtained by L. Tschugajeff<sup>3</sup> (=0.66) on strained or substituted cyclopropane derivatives (carone, thujene, etc.).

The sources of hydrocarbons were as follows:

Propane, from the Phillips Petroleum Company, c. P. grade, over 99% pure.

Propene, from the Matheson Company, c. P. grade, over 99% pure.

Cyclopropane, from the Mallinckrodt Chemical Company, over 99% pure.

Propyne, prepared by us by treating sodium acetylide with dimethyl sulfate.<sup>3</sup>

Propadiene, prepared for us by Dr. R. E. Schaad from 2,3-dibromopropane and zinc.<sup>4</sup>

Each hydrocarbon was purified carefully by fractionation on a low temperature Podbielniak column. Five cuts were taken on the plateau


(3) L. Tschugajeff, *Ber.*, **33**, 3122 (1900).

(4) G. Gustavson and N. Demjanow, *J. prakt. Chem.*, [2] **38**, 202 (1888).

TABLE I

Temp., °C.	$n_D$					$D_M$ (vac.)				
	Propane	Propene	Cyclopropane	Propyne	Propadiene	Propane <sup>5</sup>	Propene <sup>6</sup>	Cyclopropane	Propyne <sup>6</sup>	Propadiene
-20	....	....	....	1.3725	....	....	....	....	0.6670	....
-30	....	....	1.3706	1.3793	1.4137	....	....	0.6769	.6791	0.6575
-40	1.3378	1.3567	1.3769	1.3863	1.4214	0.5800	0.6002	.6886	.6911	.6699
-50	1.3447	1.3640	1.3833	....	1.4291	.5915	.6132	.7002	....	.6822
-60	1.3517	1.3712	1.3897	....	1.4368	.6029	.6258	.7119	....	.6944
-70	1.3587	1.3785	1.3960	....	1.4444	.6145	.6386	.7235	....	.7064
-80	1.3657	1.3857	1.4024	....	....	.6259	.6513	.7352	....	....
@N. b. p.	1.3392	1.3623	1.3726	1.3747	1.4168	.5829	.6100	.6807	.6698	.6624
Normal b. p.	-42.27	-47.8	-32.7	-23.34	-34.34	-42.27	-47.8	-32.7	-23.36	-34.34
$dnd/dt^\circ$ and, respectively, $dD/dt^\circ$										
	-0.000698	-0.000725	-0.000638	-0.000690	-0.000768	-0.00115	-0.00128	-0.00117	-0.00120	-0.00122

TABLE II

Hydrocarbon	Carbon skeleton	Mol. volume at N. b. p., cc.	Experimental molecular refractions, $MRL^L_D$						Extra- polated to +20°	Calculated $MRL^L_D$ (at +20°)	Temp. coeff. $MRL^L_D/^\circ C.$ (from -80° to -20°)
			@N. b. p.	-80°	-60°	-40°	-20°				
Propane	C—C—C	75.65	15.83	15.76	15.79	15.83	...	15.9	16.05	+0.0017	
Propene	C=C=C	68.98	15.30	15.15	15.24	15.34	...	15.6	15.62	+ .0047	
Cyclopropane		61.82	14.06	13.94	13.99	14.04	14.10	14.2	(13.85) <sup>a</sup>	+ .0025	
Propyne	C—C≡C	59.81	13.65	...	...	13.61	13.66	13.8	14.05	+ .0025	
Propadiene	C=C=C	60.48	15.19	15.03	15.10	15.17	...	15.3	15.12	+ .0035	

<sup>a</sup>  $3 \times CH_2$ .

corresponding to the boiling point of the pure substance. The  $n_D$  of each fraction was measured and a cut whose index did not vary over 0.0002 from adjacent cuts was used for our final measurements. These were made at temperature intervals of about 10°. For all hydrocarbons studied the  $n_D$  was a straight line function of temperature; the values of Table I were read off the line for even temperature intervals.

For measuring  $n_D$  below -50° reduced pressure was employed on liquid propene circulating through the refractometer. By this procedure we were able to measure easily  $n_D$  at as low as -75°, when using dry-ice as the cooling medium.

(5) O. Maass and C. H. Wright, *THIS JOURNAL*, **43**, 1098 (1921).

(6) F. R. Morehouse and O. Maass, *Can. J. Research*, **11**, 637 (1934).

(7) M. M. Hicks-Bruun and J. H. Bruun, *THIS JOURNAL*, **58**, 810 (1936).

(8) See J. H. Vaughn, L. F. Hennion, R. R. Vogt and J. A. Nieuwland, *J. Org. Chem.*, **2**, 1 (1937).

RESEARCH LABORATORIES  
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## Two Notes on the Deamination of Glycine in the Presence of Tyrosinase and *p*-Cresol

BY STEPHEN S. HUBARD

When collecting data which indicate that *p*-cresol oxidized in the presence of tyrosinase functions reversibly in the oxidative deamination of

glycine,<sup>1</sup> it was found that this reversibility, although considerable, is not complete. The smaller amounts of *p*-cresol do not take care of as much glycine as do the larger ones, although the amounts are greater than those theoretically equivalent, as discussed in the paper cited. Second, as found by others,<sup>2</sup> the amount of ammonia recovered is less than what corresponds to the amount of glycine deaminized. These two facts suggest that part of the oxidized *p*-cresol, and part of the glycine nitrogen, either as ammonia, or without being released as ammonia, are consumed in forming the intense dichroic coloration (red by reflected light, blue by transmitted light) characteristic of the digestion solutions. It seemed significant that the color of the one solution from which the theoretical amount of ammonia was recovered (1 mg. of *p*-cresol with 50 mg. of glycine) was green and showed no dichroism.

A few drops of dilute ammonia were added to a solution of 20 mg. of enzymically oxidized *p*-cresol; in a few hours the color of the solution had become greenish, similar to the one just mentioned. Formaldehyde is a possible *transient* end-product in the breakdown of glycine as studied here.<sup>3</sup> When two or three drops of formalin was added to another sample of oxidized *p*-cresol, a

(1) S. S. Hubbard, *J. Biol. Chem.*, **126**, 489 (1938).

(2) C. E. M. Pugh and H. S. Raper, *Biochem. J.*, **21**, 1370 (1927).

(3) F. C. Happold and H. S. Raper, *ibid.*, **19**, 92 (1925).



cherry-red color was observed eventually. However, when both formaldehyde and ammonia were added, a dichroic effect similar to that of the glycine digestion solutions developed. Like the latter, the solution became red when made strongly alkaline, and blue when made strongly acid (caustic soda, hydrochloric acid).

Preliminary efforts to isolate the colored substance that seems from the experiment just described to be formed from ammonia, oxidized *p*-cresol, and the fragment of the glycine molecule were unsuccessful, but the important thing is that we have found at least a qualitative indication of the fate of the ammonia and *p*-cresol in question, whereas before there was only speculation.

There is a discrepancy in the literature regarding resorcinol as a possible inductor in the tyrosinase-glycine reaction, which should be mentioned. Robinson and McCance<sup>4</sup> state that it functions, although there is a long "induction period," and the deamination of glycine is far from complete. Pugh and Raper<sup>2</sup> and Happold and Raper<sup>5</sup> obtained completely negative results. The clue is that Robinson and McCance used a basidiomycete as the source of the enzyme; the other workers used meal-worms and potatoes, respectively. Since Gortner has shown<sup>5</sup> that tyrosinase not only does not oxidize *m*-dihydroxylic compounds, but also is hindered by them in its oxidation of other phenols, it follows that Robinson and McCance probably did not free their preparation from a laccase which functions with resorcinol,<sup>5</sup> and which is found in the fungus they used, whereas the other workers evidently had preparations free from this laccase. The delayed action and incomplete deamination observed by Robinson and McCance are just what one would expect from a preparation in which the laccase was only a contaminant.

(4) M. E. Robinson and R. A. McCance, *Biochem. J.*, **19**, 251 (1925).

(5) R. A. Gortner, *J. Biol. Chem.*, **10**, 113 (1911).

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### Determination of the Ionization Constant of Aci-Nitroethane

BY SAMUEL H. MARON<sup>1</sup> AND THEODORE SHEDLOVSKY

In a preceding paper<sup>2</sup> were reported kinetic measurements on the rate of isomerization of ni-

(1) Present address: Department of Chemical Engineering, Case School of Applied Science, Cleveland, Ohio.

(2) Maron and La Mer, *THIS JOURNAL*, **60**, 2588 (1938).

troethane from the nitro to the aci form. To explain the kinetics of the reverse process of isomerization from aci to nitro form, a knowledge of the ionization constant of aci-nitroethane,  $\text{CH}_3\text{CH}=\text{NOOH}$ , is essential. The only value available is that given by Junell,<sup>3</sup>  $7 \times 10^{-5}$ , and obtained from kinetic measurements at 0°. The importance of this constant for kinetic purposes lends interest to a direct determination by an electrometric method.

The determination involves the complication of the instability of the ions of the aci acid, which isomerize by mutual interaction to the nitro form. Any appreciable concentration of either hydrogen ion or aci ion leads to such a rapid rate of isomerization that the *pH* of the solution varies too much during the course of a *pH* measurement for the results to have significance. This difficulty can be obviated by measuring the *pH* of solutions where the concentration of both ions is very small, *i. e.*, solutions of the aci acid practically free of salt.

A solution of barium nitroethane, 0.06341 *N*, was prepared by dissolving a weighed quantity of nitroethane in water, adding an equivalent quantity of barium hydroxide, and allowing the solution to stand for several days. This stock solution was diluted then to the desired concentrations. In these salt solutions *nearly all* the acid was liberated by the addition of a definite quantity of either hydrochloric or sulfuric acid. The *pH* of the solution was determined with a sensitive glass electrode assembly, with which it was possible to make several measurements to 0.01 *pH* unit within one minute following the acid addition. The electrode<sup>4</sup> was calibrated with a potassium acid phthalate buffer.<sup>5</sup>

The results of three measurements at 23° are given in Table I. Line (1) gives the initial concentration of barium nitroethane, lines (2) and (3) the acid added and its final concentration, and (4) the observed *pH*, while (5), (6), and (7) give the concentrations of hydrogen ion, nitroethane ion, and the undissociated acid at equilibrium. The ionization constants,  $K_i$ ,<sup>6</sup> calculated from

(3) Junell, *Svensk Kem. Tid.*, **46**, 125-136 (1934); Dissertation, University of Uppsala, 1935.

(4) Sendroy, Shedlovsky and Belcher, *J. Biol. Chem.*, **115**, 532 (1936); MacInnes and Longworth, *Trans. Am. Electrochem. Soc.*, **71**, 73 (1937).

(5) MacInnes, Belcher and Shedlovsky, *THIS JOURNAL*, **60**, 1098 (1938).

(6) In the computation of  $K_i$ , which is on a concentration basis, it has been assumed that *pH* measures hydrogen-ion concentration. We are aware of the theoretical difficulties in such an assumption. It is, however, a sufficient approximation for our purpose.

these are given in the last line, and show satisfactory agreement over better than a three-fold concentration range.

TABLE I

THE IONIZATION CONSTANT OF ACI-NITROETHANE AT 23°

(1) Init. concn. Ba nitroethane	0.04039	0.02467	0.01268
(2) Acid added	HCl	HCl	H <sub>2</sub> SO <sub>4</sub>
(3) Concn. of added acid	0.03975	0.02429	0.01226
(4) pH	3.01	3.10	3.28
(5) Concn. H <sup>+</sup> ion × 10 <sup>4</sup>	9.77	7.94	5.25
(6) Concn. nitro ion × 10 <sup>4</sup>	1.62	1.17	0.95
(7) Concn. CH <sub>3</sub> CH=NOOH	0.03877	0.02350	0.01173
(8) K <sub>1</sub> × 10 <sup>6</sup> (av. 4.09 ± 0.10)	4.08	3.95	4.25

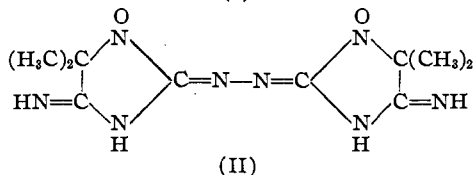
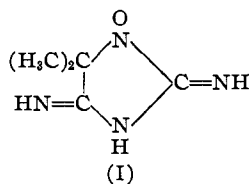
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### $\alpha$ -Hydroxylaminoisobutyronitrile—an Intermediate in the Synthesis of Porphyrexide and Porphyrindine

BY CURT C. PORTER AND LESLIE HELLERMAN

Kuhn and Franke<sup>1</sup> have shown that porphyrexide (I) and porphyrindine (II)<sup>2,3</sup> constitute, with their respective reductants, rapidly reversible oxidation-reduction systems, the characteristics of which may be measured potentiometrically.



Thermodynamically, they stand among the most powerfully oxidizing of the organic systems ( $E'_0$  at<sup>4</sup> pH 7 = +0.725 or +0.565, respectively).<sup>1</sup> The oxidants, which are unusually interesting "free radicals," have been employed recently for the estimation of certain mercaptans and of the sulfhydryl groups of certain proteins; as such, they have been used in the study of *protein denaturation*.<sup>5,6</sup>

The first step in the synthesis of these substances, involving the addition of hydrocyanic

acid to acetoxime to give  $\alpha$ -hydroxylaminoisobutyronitrile, requires the use of concentrated aqueous hydrocyanic acid and may be difficult to control. We have modified the procedure by substituting for liquid hydrocyanic acid a suitable cyanide-phosphate buffer. This device may have value also in certain other cases where concentrated hydrocyanic acid has been specified.

**Preparation of  $\alpha$ -Hydroxylaminoisobutyronitrile, (H<sub>3</sub>C)<sub>2</sub>C(NHOH)CN.**—Powdered acetoxime, 94.9 g. (1.3 moles), and 626 g. (4.6 moles) of potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) are placed in a 2-liter round-bottomed flask with ground connections for a glass stopper (which preferably carries a stopcock); water, 260 cc., is added, and the mixture is placed in an ice-bath. *Subsequent operations are conducted in an excellently ventilated hood.* To the reaction mixture is added an ice-cold solution of 112.7 g. (2.3 moles) sodium cyanide dissolved in 280 cc. of water; the addition is made rather slowly while the reagents are kept well mixed. The mixture is allowed to warm to room temperature (about 20°) during which it is shaken occasionally. A yellow surface layer will have formed. The flask with contents is conveniently placed in a large closed vessel (desiccator) and allowed to stand (under the hood) at room temperature for about eighteen hours and no longer. The whole mixture is subjected to three ether extractions (total of 640 g. of purified ether) and from the extracts without preliminary drying the hydrocyanic acid and ether are removed in a stream of clean air. The residual aqueous suspension of crystals (which may be concentrated further, if necessary, in a vacuum desiccator) is ice-cooled, transferred to a cold suction filter, freed of mother liquor, and washed thrice with 2-cc. portions of ice water.

The crude product, consisting of nitrile and unchanged oxime, is dried in a vacuum desiccator over phosphorus pentoxide; then the oxime is completely removed by means of trituration with four or five 100-cc. portions of petroleum ether (b. p. 30–35°); oxime recoverable from the extracts amounts to 20–25 g. The  $\alpha$ -hydroxylaminoisobutyronitrile is purified further, if required, by being washed with small portions of cold *n*-butanol, followed by recrystallization from ether and petroleum ether; yield 25 g.; m. p. 100°.

The procedure is reliable and convenient. Yields are not as large as those reported by Kuhn and Franke.<sup>1</sup> No difficulty is encountered in the subsequent steps of the porphyrexide synthesis, particularly if the ethanol, required as solvent in the preparation of the iminoester dihydrochloride (derived from nitrile) and of the related amidine hydrochloride, is properly dried.

For the oxidation of  $\alpha$ -hydroxylaminoisobutyramidine hydrochloride to the corresponding nitroso compound, the calculated quantity of a standard solution of sodium hypochlorite may be substituted for chlorine gas. However, in order to prevent the formation of N-chloro derivatives, enough hydrochloric acid must be present in the reaction mixture to "cover" the amidine grouping and to generate chlorine *in situ*.

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BALTIMORE, MARYLAND RECEIVED JANUARY 9, 1939

- (1) R. Kuhn and W. Franke, *Ber.*, **68B**, 1528 (1935).
- (2) O. Piloty and B. Schwerin, *ibid.*, **34**, 1863, 1870, 2354 (1901).
- (3) O. Piloty and W. Vogel, *ibid.*, **36**, 1283 (1903).
- (4) W. M. Clark and B. Cohen, *Pub. Health Repts.*, **38**, 666 (1923); reprinted in *Hygienic Lab. Bull.* No. 151, 13 (1928).
- (5) R. Kuhn and P. Desnuelle, *Z. physiol. Chem.*, **261**, 14 (1938).
- (6) J. P. Greenstein, *J. Biol. Chem.*, **125**, 501 (1938).