permanganate solution and the mixture was stirred on a steam-bath with reflux. After twelve hours the solution was decolorized and 40 g. more of potassium permanganate was added in concentrated solution. After twelve hours the addition was repeated, after which the solution was not completely decolorized by heating for four days. The excess permanganate was reduced with formaldehyde, the solution was filtered, concentrated to 200 cc. and neutralized with dilute nitric acid. Silver nitrate solution was added and the flocculent precipitate of silver salt was filtered, washed, dissolved in ammonium hydroxide and reprecipitated in three fractions by the slow addition of 1 *N* nitric acid. The second fraction was suspended in water and hydrogen sulfide passed into the mixture. The silver sulfide was removed and the solution evaporated to leave white crystals of pyridine-2,4,5-tricarboxylic acid, melting with decomposition at 242–243°. It was recrystallized from dilute hydrochloric acid from which it precipitated slowly. The air-dried sample was heated one hour at 120° to remove water of crystallization.[10]

Anal. Calcd. water of crystallization for $C_8H_5O_6N \cdot H_2O$: H_2O, 7.86. Found: H_2O, 7.90.

The anhydrous acid was analyzed for nitrogen.

Anal. Calcd. for $C_8H_5O_6N$: N, 6.63. Found: N, 6.69.

The acid was readily soluble in water and gave a dark red color with ferrous sulfate solution. It was heated to 170° without appreciable carbon dioxide evolution, a reaction that is reported[11] to be rapid above 140° for pyridine-2,3,6-tricarboxylic acid. It was apparently unaffected by boiling for two hours with acetic anhydride, giving none of the chloroform-soluble cinchomeronic anhydride which is formed[12] by this treatment of pyridine-2,3,4-tricarboxylic acid.

Isolation of a $C_{12}H_{19}N$ Base.—An intermediate fraction of bases from the distillation described above, 80 g. with $n^{20}D$ 1.4904 and b. p. 101° (20 mm.), was extracted from 500 cc. of petroleum ether in seven fractions using the apparatus of Fig. 1. Fraction 6 was converted to the picrate as described above, giving a crude picrate which was recrystallized repeatedly from dilute ethanol and dilute acetic acid to a constant melting point of 174°.

Anal. Calcd. for $C_{18}H_{22}O_7N_4$: C, 53.20; H, 5.46. Found: C, 53.20; H, 5.51.

One-half gram of the picrate was heated with concentrated ammonium hydroxide to liberate the base which was extracted in ether, dried and distilled in a semi-micro distillation apparatus. A middle fraction gave the following constants: b. p. 214° (754 mm.); $n^{20}D$ 1.4832.

Anal. Calcd. for $C_{12}H_{19}N$: N, 7.90. Found: N, 7.79.

Isolation of a $C_{13}H_{21}N$ Base.—Fraction 7 of the extraction series described above from which the $C_{12}H_{19}N$ base was obtained was converted to the picrate and recrystallized repeatedly to give yellow needles having a constant melting point of 121°.

Anal. Calcd. for $C_{19}H_{24}O_7N_4$: C, 54.28; H, 5.75; N, 13.33. Found: C, 53.87; H, 5.74; N, 13.52.

Summary

1. A sample of California petroleum bases boiling at 210–213° has been fractionated by efficient methods of extraction and distillation to yield products of sufficient purity that crystalline picrates of three new bases were obtained.

2. By a process of degradation a $C_{11}H_{17}N$ base has been identified as *dl*-2-*s*-butyl-4,5-dimethylpyridine.

3. Two other bases having molecular formulas of $C_{12}H_{19}N$ and $C_{13}H_{21}N$ were isolated in quantities too small for identification. It is believed that they are also alkylated pyridines.

AUSTIN, TEXAS RECEIVED JULY 31, 1942

(10) Weidel, *Ber.*, **12**, 410 (1879).
(11) Weiss, *ibid.*, **19**, 1310 (1886).
(12) Kirpal, *Monatsh.*, **26**, 53 (1905).

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, NO. 909]

Stereoisomeric Diphenyloctatetraenes

BY L. ZECHMEISTER AND A. L. LEROSEN

While some earlier authors[1] have denied the existence of *cis–trans*-isomers in the case of an extended conjugated double bond system, Kuhn[2] correctly summarized the situation in 1933 by the following statement: ". . . according to the available evidence a strong accumulation of double bonds does not exclude the occurrence of *cis–trans*-isomerism. That the higher diphenylpolyenes are known only in one spatial form is due to the inadequacy of the preparative methods." In the series mentioned, $C_6H_5(CH{=}CH)_nC_6H_5$, so far as we know, stereoisomeric forms have been obtained[3] only in the case $n < 3$. According to X-ray studies by Hengstenberg and Kuhn[4] the prepa-

(1) G. Wittig and W. Wiemer, *Ann.*, **483**, 144 (1930).
(2) R. Kuhn, in Freudenberg's "Stereochemie," F. Deuticke, Vienna, 1933, p. 915.
(3) F. Straus, *Ann.*, **342**, 190 (1905). An alleged white modification of diphenyl-octatetraene (H. Stobbe, *Ber.*, **42**, 567 (1909)) was later identified as stilbene by R. Kuhn and A. Winterstein (footnote 5). Accordingly, text and formulas on pp. 179–180 of the following book should be corrected: G. Egloff, G. Hulla and V. I. Komarewsky, "Isomerization of Pure Hydrocarbons," Reinhold Publishing Corp., New York, N. Y., 1942.
(4) J. Hengstenberg and R. Kuhn, *Z. Krystall. Miner.*, **75**, 301 (1930).

rations of Kuhn and Winterstein[5] represent all-*trans* forms in case of $n = 3$ or 4.

In contrast, the existence of a great number of stereoisomers has recently been demonstrated for naturally occurring polyenes, the carotenoids. For example, 14 stereoisomeric lycopenes, $C_{40}H_{56}$, have been reported, and one of them, prolycopene containing 4 or 5 *cis*-double bonds, has been isolated from natural sources.[6] A partially *cis* γ-carotene, *viz.*, *pro*-γ-carotene,[7] $C_{40}H_{56}$, also occurs in nature. Many stereoisomers can be obtained from β-carotene[8] in addition to Gillam's pseudo-α-carotene.[9]

Considering these facts a stereochemical reinvestigation of diphenylpolyenes was desirable. We have mentioned briefly[10] that some methods

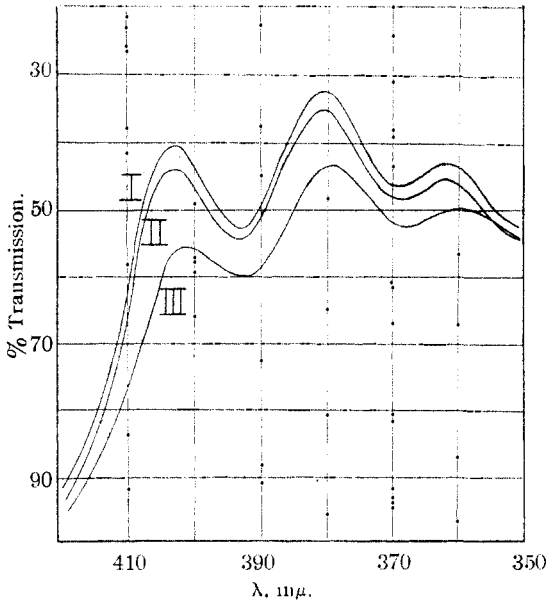

Fig. 1.—Isomerized diphenyloctatetraene in benzene. Light absorption curves of a chromatographic filtrate containing chiefly zone 2. The curves III, II, and I were taken after zero, one, and two days, respectively. Curve I remained constant on the fourth day. (Abscissa: wave length, ordinate: percentage transmission measured in the Beckman photoelectric spectrophotometer.[11])

(5) R. Kuhn and A. Winterstein, *Helv. Chim. Acta*, **11**, 87, 116, 123, 144 (1928).

(6) L. Zechmeister, A. L. LeRosen, F. W. Went and L. Pauling, *Proc. Nat. Acad. Sci.*, **27**, 468 (1941); A. L. LeRosen and L. Zechmeister, THIS JOURNAL, **64**, 1075 (1942); L. Zechmeister and R. B. Escue, *J. Biol. Chem.*, **144**, 321 (1942).

(7) L. Zechmeister and W. A. Schroeder, *Science*, **94**, 2452 (1941); THIS JOURNAL, **64**, 1173 (1942); *J. Biol. Chem.*, **144**, 315 (1942); W. A. Schroeder, THIS JOURNAL, **64**, 2510 (1942).

(8) A. Polgár and L. Zechmeister, THIS JOURNAL, **64**, 1856 (1942). The same statement is valid for α-carotene.

(9) A. E. Gillam and M. S. El Ridi, *Biochem. J.*, **30**, 1735 (1936); *ibid.*, **31**, 1605 (1937) [with S. K. Kon]; G. R. Carter and A. E. Gillam, *ibid.*, **33**, 1325 (1939).

(10) L. Zechmeister and A. L. LeRosen, *Science*, **95**, 587 (1942).

(11) H. H. Cary and A. O. Beckman, *J. Opt. Soc. Am.*, **31**, 682 (1941).

in use for the isomerization of carotenoids are applicable to synthetic polyenes.

The stereochemical homogeneity and the all-*trans* configuration of 1,8-diphenyloctatetraene (prepared according to Kuhn and Winterstein[5]) can be demonstrated chromatographically. A single zone, showing an intense light yellow fluorescence in ultraviolet light, appeared on the lime column. When, however, a benzene solution of this zone was refluxed, irradiated, or treated with iodine, or if crystals were melted for a short time, subsequent chromatography gave three well separated, fluorescing zones in each case. The upper one was identical with the single all-*trans* zone mentioned. The two other fractions possess *cis*-configurations of some double bonds which will be designated below.

It is easy to isomerize about one-sixth of the starting material, *e. g.*, the weight ratio of the three isomers was 83:15:2 from top to bottom of the Tswett column (weighed in form of the regenerated all-*trans* isomer). In some favorable cases two additional minor isomers appeared below the main zones. In this way five of the ten theoretically possible isomers were observed. They are designated as zones 1–5 (no. 1 = all-*trans* top zone).

All diphenyloctatetraenes containing *cis*-double bonds are labile. Their solutions when kept at room temperature contain a gradually increasing quantity of the all-*trans* form. The conversion becomes manifest by a decreasing light-transmission until a constant end value is reached (Fig. 1). This conversion has been followed and confirmed chromatographically.

Analogous changes were observed by starting from solid samples obtained by almost instantaneous evaporation of solutions of the zones 2–5 which had been washed through the column. Adsorption analysis showed that the fresh solution of the dry residue contained very little of the all-*trans* isomer, the quantity of which, however, rapidly increased on standing. Because of these circumstances we were unable (even working at 5°) to isolate samples of a *cis*-isomer for which the absence of the all-*trans* compound could be conclusively proved. Attempts to crystallize individual *cis*-compounds, contained in chromatographic zones, using the ordinary methods of elution and isolation so far gave only crystals of the *trans*-isomer. Combustion analysis of some isomerized, chromatographed and reconverted

samples (zones 2 and 3) proved, however, that no other change but a spatial shift within the molecule had taken place during the operations.

Another characteristic feature showing the lability of the new isomers is the very rapid reconversion of adsorbed material under the quartz lamp. In a suitably developed chromatogram those portions of zone 2 which were adsorbed on the cylindrical surface of the column, when exposed to ultraviolet light for a minute, showed on further washing a division into two fluorescing layers. The inside of the column showed only the original zone but none of the all-*trans* zone formed locally by the irradiation of the surface.

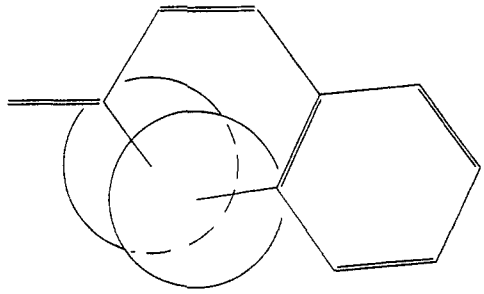

Fig. 2.—Model of one molecule end of diphenyloctatetraene (values used: C=C, 1.33 Å.; C—C, 1.46 Å.; C_6H_5—C, 1.44 Å.; C_6H_5—H, 1.08 Å.; C—H, 1.09 Å.; H-radius, 1.20 Å.; angles C=C—C and C=C—H, 124°20′.

With reference to the configuration of the individual diphenyloctatetraenes the following remarks can be made. Figure 2 shows that if an end double bond of the aliphatic chain assumes *cis*-configuration, a hydrogen atom of the nucleus, in opposition to the side chain, is spatially hindered by one of the hydrogens of the latter so that planar or approximately planar configuration becomes impossible for the molecule. The calculated deviation is about 52.5°. Therefore if the all-*trans* compound is isomerized, great preference will be given to the formation of stereoisomers in which the two end double bonds remain in *trans*-position. Instead of nine isomeric tetraenes possessing *cis*-bonds only two will be formed in any substantial quantity. We believe that these are contained in the two main zones produced by isomerization for which we suggest the following probable configurations

Zone 1 (top) *trans–trans–trans–trans*
Zone 2 (middle) *trans–cis–trans–trans*
Zone 3 (bottom) *trans–cis–cis–trans*

As with *cis*-stilbene, $C_6H_5CH{=}CHC_6H_5$, and *cis*-diphenylbutadiene, $C_6H_5(CH{=}CH)_2C_6H_5$, "hindered" isomers are also formed; this is shown by the minor zones 4 and 5 of the isomerized tetraene chromatogram. In the case of the octatetraene, isomerization is distributed between the several sterically possible types, some of which may be formed without significant steric hindrance others of which may involve definite spatial conflicts. Since the former types provide preferred configurations which may be assumed by the molecule, the sterically "hindered" types will not have the opportunity to play the prominent role which they have been found to assume in the case of stilbene (and diphenylbutadiene) in which the "hindered" isomer is without competition from other and more stable forms.

In the field of the carotenoids, according to Pauling,[12] the group >C=CH—C(CH$_3$)< constitutes a complete hindrance for a *trans–cis*-shift. The number of the expected *cis–trans*-isomers (occurring in substantial quantities) can be reduced on the basis of spatial considerations both in the class of natural and synthetic polyenes.

Acknowledgment.—The authors wish to thank Dr. R. B. Corey for valuable advice and Dr. G. Oppenheimer as well as Mr. G. Swinehart for microanalyses.

Experimental

1,8-Diphenyloctatetraene was synthesized according to Kuhn and Winterstein.[5] After recrystallization from

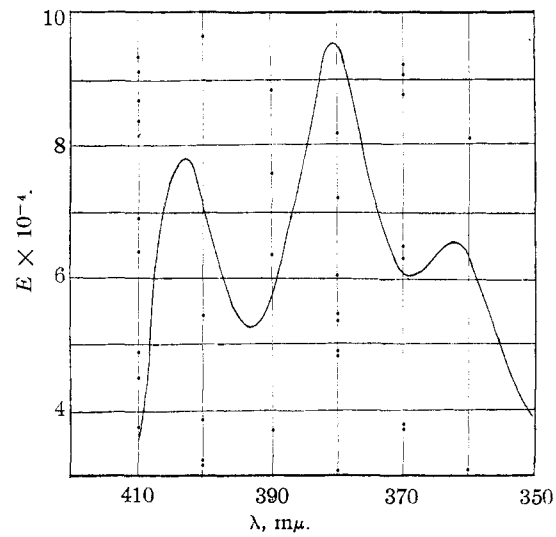

Fig. 3.—Absorption curve of all-*trans*-diphenyloctatetraene in benzene. (Abscissa, wave length; ordinate, mol. extinction coefficient.) $E = \frac{1}{Lc} \log \frac{I_o}{I}$.

(12) L. Pauling, *Fortschr. Chem. Organ. Naturstoffe*, **3**, 203 (1939).

chloroform the yellow plates melted at 235–237° (cor.). The absorption curve is given in Fig. 3.

Anal. Calcd. for $C_{20}H_{18}$: C, 92.97; H, 7.03. Found: C, 93.27; H, 7.23.

Chromatographed on calcium hydroxide (Shell brand chemical hydrate, 98% through 325 mesh) as described below, a solution of the crude or of the purified crystals showed homogeneity. The zones as well as those of stereoisomers are best located during the development on a Tswett column by means of a portable quartz lamp in the dark. Intense light yellow fluorescence appears.

1. **Isomerization in Solution by Heat.**—A solution of 0.4 g. of diphenyloctatetraene in about 200 ml. of benzene was gently refluxed for several hours, brought to 225 ml. (in order to prevent precipitation) and diluted with 3 volumes of light petroleum (b. p. 60–70°). The solution was immediately poured on the adsorbent in a percolator (40 × 16 × 8 cm.) under suction. After washing with light petroleum the chromatogram was developed with benzene–light petroleum 1:10 within a few hours. Below the main zones of unchanged all-*trans* compound a second and a smaller third zone appeared, followed by traces of two minor fractions. All zones (termed 1–5, from top to bottom) were well separated by non-fluorescing intermediate sections. Each of the main zones was cut out, eluted with benzene-methanol 3:1, quickly washed alcohol-free in a special apparatus,[13] dehydrated with sodium sulfate within a few minutes and completely evaporated *in vacuo*. Each dry residue was dissolved in dioxane and precipitated with water. The zones 1–3 yielded 0.25 g., 0.05 g. and 0.005 g. of substance, respectively. All were identified as all-*trans* compounds by mixing them together and chromatographing; only one zone appeared.

Anal. Calcd. for $C_{20}H_{18}$: C, 92.97; H, 7.03. Found (zones 1–3): C, 92.72, 93.07, 92.43; H, 7.72, 7.37, 7.94.

In other experiments the elution of the isomers formed was made with ether which was evaporated directly. Each residue when chromatographed gave only a zone of the all-*trans* form.

2. **Isomerization by Melting.**—A sealed tube containing several milligrams of diphenyloctatetraene was kept in a bath of boiling diphenyl ether (b. p. 259°) for fifteen minutes. After rapid cooling in ice-water the material was dissolved in cold benzene and diluted with light petroleum. The chromatogram showed two isomers below the main zone of unchanged starting material. A second adsorption of the lower zones revealed the presence of a strong zone of the all-*trans* isomer, formed during the operations (elution, filtration, washing, drying).

Similar results were obtained by heating diphenyloctatetraene in melted diphenyl at 140° for five hours.

3. **Isomerization by iodine catalysis** in benzene solution at 25° in the dark produced the zones 1–3 within fifteen minutes as demonstrated chromatographically.

4. **Photochemical Isomerization.**—A benzene solution containing 2 mg. of diphenyloctatetraene per ml. was divided into two parts. One part was kept in diffuse light at 25° while the other was irradiated for twelve hours with a mercury quartz lamp (4 amp. d. c., voltage drop 32 v.) in a quartz tube. A half ml. of the irradiated solution, chromatographed on a column (25 × 1.7 cm.), after suitable development showed the following zones from top to bottom (the figures on the left side denote width of zones, in mm.):

10 *zone 1* (all-*trans*), strongly fluorescing
5 dark interzone
20 *zone 2*, fluoresced more weakly than 1, contained much less material
5 dark interzone
2.5 *zone 3*, well defined, similar to 2 in fluorescence
2 dark interzone
2 *zones 4 and 5*, not well differentiated (traces), easily washed into the filtrate.

The non-irradiated portion showed a similar chromatogram but a much smaller fraction of the adsorbed material was contained in the zones 2–5 than after irradiation.

In another set of experiments the modifications *a* and *b* were made:

a. The irradiated solution was poured on a short column prepared in a Gooch funnel (8 × 4 cm.) which was attached to a side neck of a three-neck flask (1 liter). Three fluorescing layers were visible. The two lower zones were slowly washed into the flask with benzene-light petroleum (1:1). The flask was immersed in hot water; each drop of the filtrate was rapidly evaporated by a current of air under reduced pressure. The whole procedure was followed by an ultraviolet lamp in the dark. In this way a solid residue was obtained consisting of a mixture of all-*trans* diphenyloctatetraene and a preponderant quantity of its steroisomers. A subsequent chromatogram showed a narrow top zone 1 of the all-*trans* form while zone 2 was four times broader. It was followed by a smaller layer, all separated by non-fluorescing sections.

A portion of the solid mixture was investigated spectrophotometrically in benzene solution, immediately and after twenty-four hours of standing. It shows considerably decreased transmission, corresponding to the formation of more *trans*-isomer.

b. In other experiments the first portion of the chromatographic filtrate was rechromatographed and the two lowest isomers were washed through with pure benzene. The light absorption curve was determined immediately as well as after one, two and three days of standing at room temperature. The transmission decreased considerably in the course of the first day, less in the second day and thereafter it remained constant. An equilibrium had been reached in which the all-*trans* form was predominant (Fig. 1).

Summary

Ordinary (all-*trans*) 1,8-diphenyloctatetraene, $C_6H_5(CH{=}CH)_4C_6H_5$ when heated, irradiated or treated with iodine in benzene solution is partially converted into two main stereoisomers which can also be obtained by melting of crystals. These labile isomers are adsorbed below the unchanged, all-*trans* compound in the lime column. Their solution re-isomerize spontaneously at room temperature. This has been followed chromatographically and photometrically. A similar but

(13) A. L. LeRosen, *Ind. Eng. Chem., Anal. Ed.*, **14**, 165 (1942).

more rapid change occurs on irradiation of adsorbed material. The configurations *trans-cis-trans-trans* and *trans-cis-cis-trans* are suggested for the new isomers mentioned. As two minor isomers also appear, five out of the ten possible steric forms of diphenyloctatetraene have been observed. It is shown that the formation of substantial quantities of certain isomers is prevented by spatial conflicts.

PASADENA, CALIFORNIA RECEIVED AUGUST 27, 1942

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF RADCLIFFE COLLEGE AND HARVARD UNIVERSITY AND THE DEPARTMENT OF BIOLOGICAL CHEMISTRY OF THE HARVARD MEDICAL SCHOOL]

Does the Parathyroid Hormone Influence Phosphatase Activity?

BY THOMAS R. WOOD AND WILLIAM F. ROSS

It is well established that serum phosphatase is markedly increased in rickets, hyperparathyroidism and other diseases characterized by lesions in the bones.[1,2,3] It has even been suggested that the action of phosphatases on organic phosphate compounds in bone "is the factor that controls the direction and intensity of calcification in bone."[3] This concept is supported by the increased serum phosphatase activity after parathyroid hormone injection,[4] and by similar changes in bone phosphatase of rats following injection of the hormone.[5,6]

The question arises, as to whether there is an *in vitro* influence of the parathyroid hormone on the activity of phosphatase. Such an effect, if reasonably pronounced, would afford a simple, economical, and rapid assay method for the hormone, which is sorely needed at the present time. Heymann[7] reported that glycerophosphatase and hexosediphosphatase of bone are inhibited by parathyroid extract, but Bakwin and Bodansky,[8] drew the opposite conclusion, that rat and cattle bone phosphatases are not affected. Both of the above studies involved the use of very small amounts of impure parathyroid preparations.

The effect of a very active parathyroid extract[9] on the hydrolysis of glycerophosphate by a kidney phosphatase preparation has therefore been investigated. The hormone concentrate had a nitrogen potency of 300 units per mg. of nitrogen, thus being three times as active as any preparation hitherto reported,[10] and many times more active than those used in the phosphatase studies referred to above.[7,8] At the same time experiments were carried out in which two other proteins, thrice-crystallized egg albumin and crystalline, carbohydrate-free horse serum albumin[11] were substituted for the hormone. Other conditions such as *p*H, temperature, and magnesium ion concentration were identical in all experiments.

The data obtained from these studies give the curves of Fig. 1. Under our conditions each of the three proteins has an activating effect upon phosphatase, and the parathyroid hormone is not unlike the other two substances in any respect. Characteristic of the curves is the tendency to approach a constant maximum, which is not followed by a subsequent decline in activity.

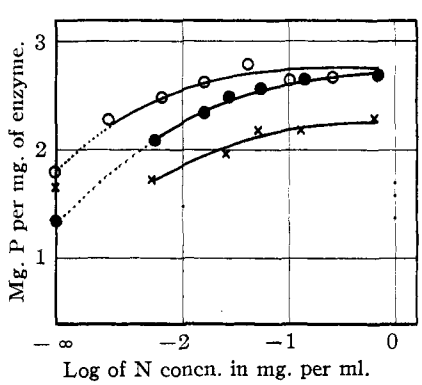

Fig. 1.—The *in vitro* effect of parathyroid hormone (O), horse serum albumin (●), and egg albumin (X) on phosphate liberation by kidney phosphatase.

This behavior of phosphatase recalls its activation by other nitrogen containing compounds, such as ammonia, amino acids and veronal. The activity in the presence of these substances, however, passes through a maximum and then decreases.

(1) Roe and Whitman, *Am. J. Clin. Path.*, **8**, 233 (1939).
(2) Bodansky and Jaffe, *Arch. Internal Med.*, **54**, 88 (1934).
(3) Peters, Robbins and Lavietes, *Ann. Rev. Biochem.*, **5**, 295 (1936).
(4) Page and Reside, *Biochem. Z.*, **226**, 273 (1930).
(5) Williams and Watson, *Endocrinology*, **29**, 250 (1941).
(6) Page, *Biochem. Z.*, **223**, 222 (1930).
(7) Heymann, *ibid.*, **227**, 1 (1930).
(8) Bakwin and Bodansky, *Proc. Soc. Exptl. Biol. Med.*, **31**, 64 (1933).
(9) Ross and Wood, *J. Biol. Chem.*, **146**, 49 (1942).
(10) Collip and Clark, *ibid.*, **66**, 133 (1925).
(11) McMeekin, THIS JOURNAL, **61**, 2884 (1939).