catalyst was removed and the solution passed over another IRA-400 column to convert the methochloride to the methohydroxide. The solvent was carefully removed under reduced pressure, and the residue transferred to the molecular still and heated to 75° at 1–2 mm. A colorless liquid distilled at this temperature, $n^{20}D$ 1.4810, infrared max. 10.08 and 11.01 μ.

Anal. Calcd. for $C_{12}H_{21}N$: C, 80.38; H, 11.81. Found: C, 80.33; H, 11.89.

Its picrate, recrystallized from alcohol, melted at 170.4–171.2°.

Anal. Calcd. for $C_{18}H_{24}N_4O_7$: C, 52.93; H, 5.92. Found: C, 53.23; H, 6.11.

Its methiodide crystallized from alcohol–ethyl acetate and gave the same range after repeated recrystallization, m.p. 186–200°.

Anal. Calcd. for $C_{13}H_{24}NI$: C, 48.60; H, 7.52. Found: C, 48.78; H, 7.55.

1-Vinyltetrahydropentalene (XIX).—A methanolic solution of 425 mg. (0.0014 mole), of the methiodide of XVI was passed over an IRA-400 column (hydroxide form), the solvent distilled carefully under reduced pressure, and the residue transferred to the molecular still and heated to 100–130° at 15 mm. The distillate was taken into pentane, washed with dilute hydrochloric acid, dried and the pentane evaporated leaving 74 mg. (0.00056 mole, 41%) of the hydrocarbon, ultraviolet max. 247 mμ.

Anal. Calcd. for $C_{10}H_{12}$: C, 90.85; H, 9.15. Found: C, 89.72; H, 9.19.

The picrate of trimethylamine was formed from the products in the Dry Ice trap of the distillation system, m.p. 216–223°, from alcohol.

Attempted Dehydrogenation of XII.—A stream of nitrogen was passed over the 1-vinyltetrahydropentalene (XII) and the vapors were passed through a tube packed with 5% palladinized asbestos and 10% palladium-on-charcoal at 300–310°. The product was a deep blue oil which collected at the end of the tube. The oil was washed out with pentane and chromatographed on alumina in pentane. The first band gave about 30 mg. of a yellow liquid after evaporation of the solvent. Its infrared and ultraviolet spectra were different from those of the starting material (ultraviolet max. 252 mμ).

Anal. Calcd. for $C_{10}H_{12}$: C, 90.85; H, 9.15. Found: C, 90.64; H, 9.39.

The second fraction gave 3.5 mg. of a deep blue partially crystalline material. No infrared curve was obtained. Its ultraviolet spectrum (arbitrary extinction scale) was essentially identical with that of azulene, showing maxima at 240, 270, 274, 282, 328, 342 and 354 mμ.

Attempted Dehydrogenation of XIX.—A stream of nitrogen was passed slowly over 40 mg. of XIX and the vapors were passed through a tube containing 5% palladinized asbestos at 300–310°. Blue crystals began to form at the end of the tube, but these soon disappeared in the wake of a deep green oil. The product was washed out of the tube with pentane and passed over an alumina column. Elution with pentane gave a pale blue band, which passed readily into the filtrate to yield less than 1 mg. of material, ultraviolet max. 240, 281, 342–348 mμ. The bright green band was eluted with benzene. Evaporation of the solvent gave 3 mg. of a deep green oil. This compound gave an ultraviolet spectrum almost identical with that of the blue material.

ROCHESTER, NEW YORK

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF BRANDEIS UNIVERSITY]

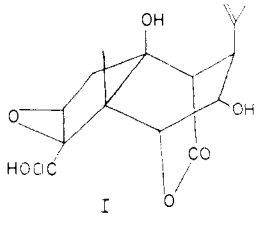
Picrotoxin. V. Conformational Analysis and Problems of Structure[1]

BY HAROLD CONROY

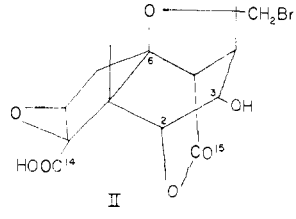
RECEIVED MAY 7, 1957

Conformational analytic principles are employed to account for reactivities in the picrotoxinin series. Structural expressions are proposed for picrotoxinin itself, and for those of its transformation products known as bromopicrotoxinin, picrotoxic acid, Schlittler's "Compound C," picrotoxinindicarboxylic acid and its esters.

In part III[1c] of this series we outlined the development of structural formulas for the α-picrotoxininic and bromopicrotoxininic acids (I and II). This and subsequent papers will show that the conclusions presented there can be broadened to include all other substances in the picrotoxin group and most particularly, the parent naturally occurring compounds, picrotoxinin and picrotin.



I



II

β-Bromopicrotoxininic acid (II) is prepared[2,3] by the action of aqueous alkali upon the dilactone, β-bromopicrotoxinin, but the process does not involve merely the opening of a lactone to the corresponding hydroxy-acid; we see that the dilactone formally derived from II by abstraction of the elements of water from the C-14 carboxyl and the C-3 hydroxyl is sterically impossible. The infrared spectra[1a,1c] suggest that the brominated derivative contains two somewhat strained five-membered lactones (absorption near 1790 cm.$^{-1}$) neither of which is present in the acid (1742 and 1705 cm.$^{-1}$ or 1769 and 1745 cm.$^{-1}$; *cf.* ref. 1c, footnote 13); moreover the bromodilactone shows no hydroxyl absorption in the infrared. *The formulation for β-bromopicrotoxinin is given in III.*[4] The action of alkali upon III, in striking contrast to the result with picrotoxinin itself (*vide infra*), then must involve transesterification, probably through the intermediate IV; the driving force for the formation of this lactonic acid (II) with one equivalent[6] of base is considered to be due both to the more effective inductive sta-

(1) The previous articles in this series appear in (a) THIS JOURNAL, **74**, 491 (1952); (b) **74**, 3046 (1952); (c) **79**, 1726 (1957); (d) *Chemistry & Industry*, 704 (1957). In a brief preliminary report ((e) THIS JOURNAL, **73**, 1889 (1951)) structures for picrotoxinin and picrotoxic acid were proposed on the basis of the more limited evidence then available.

(2) R. J. Meyer and P. Bruger, *Ber.*, **31**, 2985 (1898).

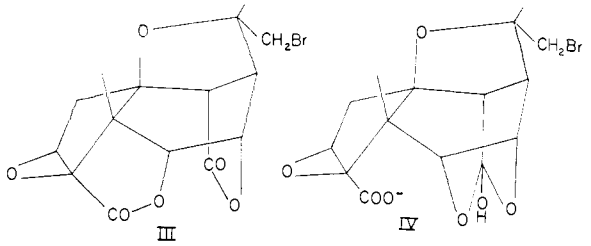
(3) P. Horrmann, *ibid.*, **45**, 2090 (1912); *ibid.*, **46**, 2793 (1913); *Ann.*, **411**, 273 (1916).

(4) The reservations of footnote 11, ref. 1c, with regard to the precise formulation of the β-bromoether linkage still apply. We do not find the arguments of the New Zealand group[5] convincing on this score.

(5) R. B. Johns, S. N. Slater, R. J. Woods, D. Brasch and R. Gee, *J. Chem. Soc.*, 4715 (1956).

(6) The reaction with excess of hot aqueous base upon II or III leads ultimately to opening of the oxide ring; *cf.* ref. 1c.

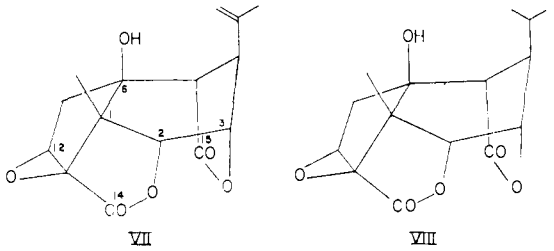
bilization of the C-14 carboxylate ion and to conformational effects. In II the cyclohexane ring is anchored in the *boat* form by *cis* fusion to the cyclopentane and by the β-bromoether linkage but the transition from the distorted *half-boat* of III allows



III IV

strain relief to a marked degree. The postulated difference in the arrangement of the lactonic systems of II and III has proved useful in accounting for the reactions[7] of these compounds with lithium aluminum hydride where the lactonic acid and the dilactone actually yield different products.

Picrotoxinin itself gives III upon bromination and can be formed from III upon zinc dust reduction; *picrotoxinin then is to be represented by the expression VII*, which alone serves to integrate these and the host of other singular characteristics so far encountered.



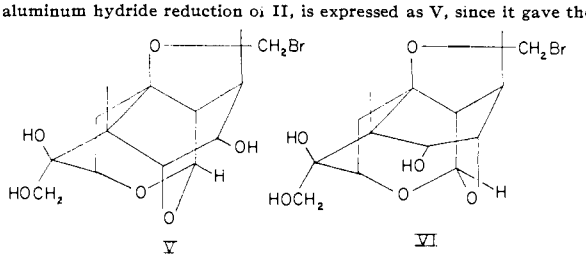
VII VIII

It will be seen that the isopropenyl group in VII is held rigidly within bonding distance of the tertiary hydroxyl. In addition to Baeyer strain and non-bonded repulsions at the eclipsed centers, C-1, C-2 and C-6, there should exist appreciable repulsive interactions involving this *axial* isopropenyl function and tending to move it into a more favorable equatorial position; it will be shown that such elementary considerations go far as bases for understanding of the special and ofttimes otherwise perplexing chemistry of this group. We can emphasize again that the abnormal unreactivity of the oxirane system is due to the proximity of the C-15 lactone bridge to the rear of the oxide ring and the protection against external nucleophilic attack thereby afforded.

Dihydropicrotoxinin (or α-dihydropicrotoxinin), prepared by catalytic hydrogenation[8,9] of VII with a platinum catalyst, is represented as VIII; like VII it does not acetylate under the usual conditions with acetic anhydride, alone or with pyridine. However, according to the New Zealand group,[10] dihydropicrotoxinin forms a *diacetate*, m.p. 245°, 259° (cor.), with acetic anhydride in the presence of ferric chloride. Since such behavior seemed extraordinary for a substance containing only one acylable group we were led to repeat the preparation of this acetyl derivative. The substance we obtained is identical (as shown by infrared and mixed m.p.) with a sample kindly furnished by Professor Slater, but our material appears instead to be a monoacetate[11,11a] as expected.

The chemistry of picrotoxic acid, prepared[3] by the action of alkali or hot, dilute acid upon picrotoxinin, provides excellent examples of conformational effects. Clearly the existence of two different isomeric monobasic acids $C_{15}H_{18}O_7$, *i.e.*, α-picrotoxininic acid (II) and picrotoxic acid, meshed well with the dilactone proposal when it was imagined that each acid was derived by saponification of a different lactone. We have shown from infrared studies that the situation with regard to α-picrotoxininic acid is actually not quite that simple, but the infrared spectrum of picrotoxic acid methyl ester shows,[1a] besides the ester band at 1730 cm.$^{-1}$, a five-membered lactone peak at 1795 cm.$^{-1}$ which is apparently identical with one of the two (1795

(7) *Cf.* refs. 5 and 1d. The product, $C_{15}H_{21}O_6Br$, formed in lithium aluminum hydride reduction of II, is expressed as V, since it gave the



V VI

same ketone, $C_{15}H_{17}O_5Br$, m.p. 200–201°, as obtained by lead dioxide oxidation of dihydro-β-bromopicrotoxininic acid.[1c] However lithium aluminum hydride reduction of III gave no V, but instead an isomer (VI) together with a substance, $C_{15}H_{23}O_6Br$, for which a plausible formulation has been given.

(8) D. Mercer and A. Robertson, *J. Chem. Soc.*, 288 (1936); R. W. H. O'Donnel, A. Robertson and J. C. Harland, *ibid.*, 1261 (1939).

(9) S. N. Slater, *ibid.*, 806 (1949).

(10) J. C. Benstead, H. V. Brewerton, J. R. Fletcher, M. Martin-Smith, S. N. Slater and A. T. Wilson, *ibid.*, 1042 (1952).

(11) Calcd. for $C_{19}H_{22}O_8$: C, 60.31; H, 5.82; $2CH_3CO$–, 22.8. Calcd. for $C_{17}H_{20}O_7$: C, 60.70; H, 5.99; $1CH_3CO$–, 12.8. Found (ref. 10): C, 60.1, 60.15; H, 5.87, 5.85; CH_3CO–, 21.3. Found (our sample, analyses by Dr. S. M. Nagy of M.I.T.): C, 60.31; H, 6.01; CH_3CO–, 13.6. The infrared spectrum (KBr disk or Nujol) shows lactone peaks at 1773 and 1802 cm.$^{-1}$ as well as an acetate band at 1736 cm.$^{-1}$; the integrated intensity of the acetate band is actually slightly lower than that of either lactone peak, not twice as high, as for a diacetate. The great similarity of the fingerprint regions of the spectra of dihydropicrotoxinin and its acetate exposes the simplicity of the relation between them, while we find, as has been pointed out,[5] that there is no hydroxyl absorption in the spectrum of the acetate.

This information, and many other data pointing to the presence of only one hydroxyl group in picrotoxinin, are in disagreement with the more recent active hydrogen results,[5] provided these are interpreted at face value. The disagreement with the earlier determinations[3] is considerably less. We do not believe that the results properly reflect the number of hydroxyl groups present not only because the outcome is inconsistent with our structural hypotheses but because it is also inconsistent with any other structural hypothesis that one might imagine. The analyses are internally at odds if compounds with a clear relationship to one another do not give the expected difference between numbers of active hydrogen atoms. For example, the Zerevitinov determinations reported in ref. 5 give, for picrotoxinin, 2.12; for dihydropicrotoxinin, 3.96; for "dihydropicrotoxinin diacetate," 4.02 active hydrogens.

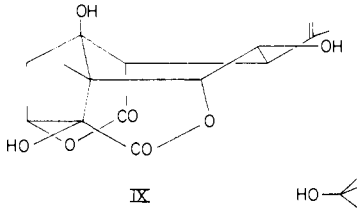
Since we have not attempted to repeat the work, we do not question the accuracy or reproducibility of these numbers, but only the use to which they might be put; it seems clear enough that hydrogen atoms other than those directly attached to oxygen have been removed under the conditions of the determinations.

(11a) NOTE ADDED IN PROOF.—In a letter dated June 6, 1957, Prof. S. N. Slater has very kindly informed us that after further acetyl determinations on his own specimens of dihydropicrotoxinin acetate he agrees that the previous figure (ref. 10) is incorrect and that the compound in question is a monoacetate.

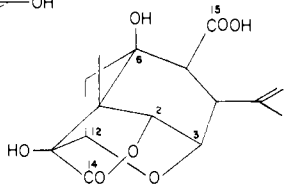
and 1776 cm.$^{-1}$) of picrotoxinin. Therefore in 1951 we were led to propose a formula[1e] (XIII, *vide infra*) in which the C-15 lactone of picrotoxinin was merely saponified to the hydroxy-acid; this formula now requires reconsideration.

A substance known as picrotoxinindicarboxylic acid, $C_{15}H_{20}O_8$, can be obtained,[3] along with picrotoxic acid, by the action of excess alkali upon picrotoxinin. The alarming feature is that more alkali does not convert picrotoxic acid to the dibasic acid! The dilemma is further compounded when we recognize that the dicarboxylic acid shows not the slightest tendency to relactonize to picrotoxic acid, and it is clear that irreversible changes must accompany the formation of either picrotoxic acid or the diacid, or both.

A clue to the constitution of picrotoxic acid will be found in the fact that the compound contains an *alcoholic* hydroxyl group sufficiently acidic to be methylated by diazomethane; this was shown by the conversion[12] of the acid to a methyl ester-methyl ether, identical with Schlittler's "Compound C," produced[13] directly from picrotoxinin with diazomethane in the presence of alkali.[14] An hydroxyl group located in close proximity to a carbonyl function meets the requirement[15] and generation of the system $-CO-\underset{|}{\overset{|}{C}}-OH$ in picrotoxic acid implies simply an attack upon the oxirane ring at C-12 by some internal nucleophilic grouping. A precedent for this is set in the formation[1c] of apopicrotoxininic dilactone (IX), isomeric with picrotoxic acid and also an α-hydroxy-lactone, but if the oxirane has been destroyed in picrotoxic acid then the functionality requires a new oxidic linkage, rather than a new lactone, to have taken its place. The problem then having been reduced to simple terms, we can discern an oxygen atom, *viz.*, at C-3 and liberated in the saponification of the C-15 lactone, in the proper steric situation for the displacement; *the revised formula for picrotoxic acid is X*, and the characterization of the change as irreversible is seen to be no exaggeration.



IX



X

The structure X is satisfactory in all other ways. It retains one of the five-membered lactones originally present in picrotoxinin. It indicates no sensitivity to periodic acid oxidation.[1e] Picrotoxic acid can be recovered from its solution in excess alkali even though conductometric titrations[10] show that the lactone neutralizes a second equivalent of base. The lactone system must be stable and so rigid that the corresponding hydroxy-acid spontaneously recloses. This feature is eminently well expressed in X, since examination of a model of the open form indicates that C-14 and the C-2 hydroxyl steadfastly remain within bonding distance despite any normal conformational changes.

The most significant aspect of structure X is that the corresponding derivative with the β-bromoether bridge (as in II) is sterically *impossible*. Fortunately enough no such substance is known.[16] Because the tertiary C-6 hydroxyl in X is *equatorial* with respect to the cyclohexane *boat*, it can no longer bond with the isopropenyl. And in the converse we have for the first time a convincing explanation for the profound effect of prior bromination upon the course of reaction of these compounds with alkali, and otherwise. When (because of the restrictions imposed by the β-bromoether linkage) the C-6 oxygen, the C-15 carboxyl, the isopropyl residue and the C-2 oxygen are all *axial*, the C-3 oxygen is *equatorial* and cannot approach C-12; the system tends to revert to the "picrotoxininic" series. But if conformations are not frozen by bromination, the large carboxyl and isopropenyl groups naturally tend to occupy *equatorial* positions when they can get them, *i.e.*, when the C-15 lactone is opened.[17] This permits the C-3 oxygen to retain the *axial* arrangement (on the new boat) necessary for displacement at the rear of the epoxide: the "picrotoxic" series results.

Returning to the problem of picrotoxinindicarboxylic acid, we find the following facts of consequence: (i) The diacid reacts readily with neutral periodate[1e] and so cannot contain the new and stable oxide bridge of picrotoxic acid. (ii) Dilute sodium methoxide in absolute methanol transforms picrotoxinin to dimethyl picrotoxinindicarboxylate,[3,12] which likewise reacts with periodate. (iii) The diacid has not been converted to picrotoxic acid and apparently shows little or no tendency to relactonize or otherwise revert to a monobasic acid. (iv) Although picrotoxic acid is unchanged, α-picrotoxininic acid is converted by excess alkali to the diacid in comparatively high yield and here, in contrast to the preparation of the diacid directly from picrotoxinin, no picrotoxic acid is formed.[3] (v) Diazomethane does not transform the dimethyl ester to any corresponding methyl ether under such conditions[12] that methyl picrotoxate is converted to "Compound C." (vi) The infrared spectra of the diacid and its diester are consistent with the

(12) J. C. Benstead, R. Gee, R. B. Johns, M. Martin-Smith and S. N. Slater, *J. Chem. Soc.*, 2292 (1952).

(13) M. Sutter and E. Schlittler, *Helv. Chim. Acta*, **33**, 902 (1950).

(14) We had independently suspected that "Compound C" is closely related to methyl picrotoxate from the virtual identity not only of the infrared carbonyl regions but also of the very characteristic fingerprint absorptions of the two compounds.

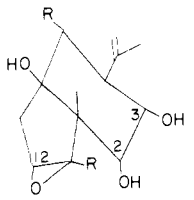
(15) *Cf.* B. Eistert in "Newer Methods of Preparative Organic Chemistry," Interscience Publishers, Inc., New York, N. Y., 1948, p. 520.

(16) Certain publications (*e.g.*, ref. 10) have stated that picrotoxic acid does not undergo the characteristic bromination of picrotoxinin and α-picrotoxininic acid without leaving clear just what does happen. The situation will probably not be strictly analogous to that of bromoapopicrotoxininic dilactone,[1c] which is strained but certainly not impossibly so.

(17) The rigidity enforced by the newly formed oxide ring in X, when coupled with the loss of steric strain due to the hitherto *axial* carboxyl and isopropyl residues and with the fact that C-15 will be present as a carboxylate ion in basic media is seen as an explanation of the non-occurrence of dealdolization with X, in contrast to the behavior of α-picrotoxininic acid (I).

presence of carboxyl or carbomethoxyl as the only carbonyl functions.

The diacid and its diester must be XI and XII. The formation of these substances is regarded merely as the twofold hydrolysis or methanolysis of the dilactone, etc., but the non-conversion of XI to picrotoxic acid, together with the point iv above, requires special comment, once again in terms of a

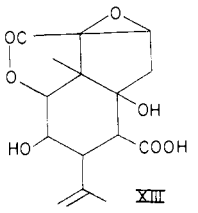


XI, R = COOH
XII, R = COOMe

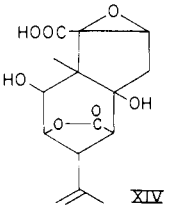
conformational argument. We note that XI and XII are exceptions to the series discussed hitherto, for the cyclohexane ring can be represented in the *chair* form, where, especially in consideration of the conjoined *equatorial* character of the bulky carboxyl and isopropenyl functions, non-bonded interactions should be minimal. But when this *chair* is attained the C-3 hydroxyl is similarly *equatorial* and thus not properly disposed for epoxide opening (*vide supra*); it is necessary to assume that the barrier for reversion to the *boat* (as in X), with an *axial* C-3 hydroxyl, is sufficient to preclude reaction under the conditions. In this view the outcome of competition in formation of picrotoxic acid and of the diacid depends upon whether the C-3 alkoxide ion survives in the *axial* conformation long enough to displace at C-12[18]; we find it satisfying to note then that the methanolysis product ratio, methyl picrotoxate/dimethyl picrotoxinindicarboxylate, increases markedly with increasing methoxide ion concentration.[7] Provided proton exchange with the solvent is rapid compared to other processes, the instantaneous concentration of the intermediate C-3 *axial* alkoxide (of XIII) will be higher when there is more base. Obviously enough, moreover, the hydrolysis of α-picrotoxininic acid (I) to the diacid XI is not accompanied by formation of picrotoxic acid because the C-3 hydroxyl is already *equatorial* in I and need never become *axial* in the course of the change involved.

Acknowledgment.—We wish to thank Miss Beatrice Bonné for technical assistance and S. B. Penick & Co. for generous gifts of picrotoxin.

(18) The reaction course (VII → X *vs.* VII → XI) may well also depend upon the state of the C-14 lactone at the time the C-3 hydroxyl is liberated, for it is attractive to consider that the C-14 lactone can remain intact in the production of picrotoxic acid (*via* the anion of XIII), but the *chair* form of the diacid XI will surely be more easily attained after saponification of this lactone. The acid-catalyzed preparation of picrotoxic acid from VII involves XIII, protonated at the epoxide. We observe further: (i) that picrotoxinindicarboxylic



XIII



XIV

acid (XI) does not relactonize to XIII because in the diacid the C-2 hydroxyl is *axial* upon the *chair*, at some distance from the *quasi-equatorial* C-14 carboxyl, so that relactonization would require reversion to a *boat*, or *half-boat*, with attendant increase in strain; (ii) that spontaneous reconstitution of the lactone (XIV) is not observed for similar reasons; (iii) that no compounds to which the structures XIII and XIV can be attributed are described in the literature.

WALTHAM, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, NEW YORK STATE PSYCHIATRIC INSTITUTE AND COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

Synthesis of Several Phosphorylated Derivatives of Dihydrosphingosine[1]

BY BENJAMIN WEISS

RECEIVED MAY 18, 1957

Dihydrosphingosine-1-phosphate and 3-O-methyldihydrosphingosine II-1-phosphate were prepared from sphingosine and 3-O-methylsphingosine II, respectively, by the following sequence of reactions: (1) hydrogenation to the dihydro form, (2) carbobenzoxylation to block the amino group, (3) phosphorylation with diphenylphosphoryl chloride and (4) removal of the protective groups by catalytic hydrogenolysis. Dihydrosphingosine-1,3-diphosphate was obtained by reduction of N-carbobenzoxy-1,3-bis-(diphenylphosphoryl)-sphingosine which was prepared by successive carbobenzoxylation and diphenylphosphorylation of sphingosine. The dihydrosphingosine-1,3-diphosphate had to be separated from sphingine-1-phosphate which resulted from the hydrogenolysis of the allylic carbon–oxygen bond of N-carbobenzoxy-1,3-bis-(diphenylphosphoryl)-sphingosine.

Since the sphingolipide fraction of monkey brain is labeled after perfusion with either 1-C^{14}-acetate or 1-C^{14}-octanoate,[2] it is of interest to study *in vitro* the synthesis of glyco- and phosphosphingosides by brain tissue so that greater insight may be gained into the intimate mechanisms of sphingolipide metabolism. In order to pursue such studies, it was necessary to prepare derivatives of sphingosine to be used as substrates. Sphingosine-1-phosphate and dihydrosphingosine-1-phosphate were particularly desirable but, after many attempts, the synthesis of sphingosine-1-phosphate was not achieved. It is hoped, however, that in the forthcoming enzymatic investigations the dihydrosphingosine-1-phosphate which was prepared may replace the unsaturated monophosphate ester until such time as it becomes available. Sphingosine phosphate has been identified by means of solvent extraction and paper chromatog-

(1) This investigation was supported in part by research grant No. B-344 (C5 and C6) from the Institute of Neurological Diseases and Blindness of the National Institutes of Health, Public Health Service.

(2) B. Weiss, *J. Biol. Chem.*, **223**, 523 (1956).