the Biology Division of the California Institute of Technology and the support and encouragement received from Dr. G. W. Beadle was greatly appreciated. Those portions of the work carried out at Brookhaven National Laboratory and the University of California were done under contract with the Atomic Energy Commission.

UPTON, L. I., N. Y.
WEST LOS ANGELES, CALIF.
CAMBRIDGE, MASS.

[CONTRIBUTION FROM THE CHEMICAL RESEARCH DIVISION, U. S. ARMY CHEMICAL WARFARE LABORATORIES]

The Stereochemistry of Asymmetric Phosphorus Compounds. I. The Resolution of O-Ethyl Ethylphosphonothioic Acid

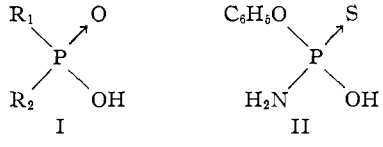
BY HERBERT S. AARON, THOMAS M. SHRYNE AND JACOB I. MILLER

RECEIVED AUGUST 9, 1957

The resolution of O-ethyl ethylphosphonothioic acid represents the first resolution of an organophosphorus acid. This acid has been resolved with both quinine and brucine in acetone solution; the (−)-antipode forms the more insoluble quinine salt, the (+)-antipode the more insoluble brucine salt. The acid may be distilled or dissolved in the presence of excess acid or base without racemization. Since a resolved acid of this type may be used for the synthesis of a host of asymmetric organophosphorus compounds, it becomes a most useful tool for application to stereochemical studies in the organophosphorus field.

The fact that a phosphorus atom containing four dissimilar substituents possesses an asymmetric configuration was first proved in 1911 by the isolation of (+)-methylethylphenylphosphine oxide.[1] In more recent years, other types of organophosphorus compounds also have been resolved, the literature of which is summarized in a recent[2] report of the resolution of an organophosphorus ester.

The literature also contains a number of reports[3] of unsuccessful attempts to resolve tetracovalent organophosphorus acids (I). Caven early suggested[3a] that a tautomeric shift of the proton in compounds of this type would lead to a rapid racemization; Ephraim, however, was the first to recognize[3d] that such acids are theoretically incapable of resolution by virtue of the fact that their anion (*i.e.*, the form in which the acid would be resolved by an optically active base) is optically inactive.

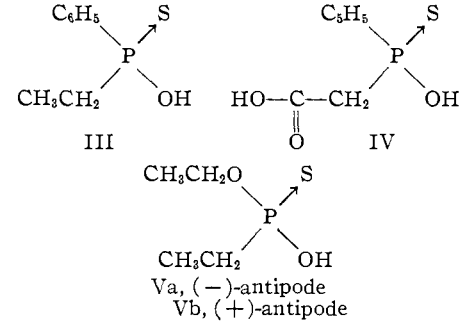


I II

R = CH_3, C_6H_5, C_6H_5O, NH_2, etc.

Ephraim then replaced one of the oxygen atoms with a sulfur atom in order to obtain an acid (II) which he postulated would possess an asymmetric anion. His attempts to resolve this acid with cinchonine, however, were unsuccessful; a crystalline salt could not be obtained. Arbuzov and Kamai[4] also took this approach. While they describe the synthesis of both phenylethylphosphinothioic acid (III) and phenyl-(carboxymethyl)-phosphinothioic acid (IV), mention is made only of attempts to resolve IV. The resolution was unsuccessful; a crystalline alkaloid salt could not be obtained here, either.

A recent communication[5] from these laboratories recorded the resolution of O-ethyl ethylphosphonothioic acid[6] (V). This disclosure constituted, therefore, the first report of the resolution of an organophosphorus acid. This paper presents a more detailed description of this work.



O-Ethyl ethylphosphonothioic acid (V) readily forms crystalline quinine and brucine salts, both of which have been used to effect a resolution of the acid. Both enantiomorphs Va and Vb have been obtained from the quinine resolution. Only the less soluble diastereoisomeric salt was recovered from the brucine resolution; no attempt was made to obtain the more soluble form from this system. The two enantiomorphs show a reverse order in the acetone solubilities of their respective diastereoisomeric quinine and brucine salts; the more insoluble diastereoisomeric salts from both systems

(1) J. Meisenheimer and L. Lichtenstadt, *Ber.*, **44**, 356 (1911).

(2) (a) D. M. Coyne, W. E. McEwen and C. A. VanderWerf, THIS JOURNAL, **78**, 3061 (1956); see also (b) K. L. Marsi, C. A. VanderWerf and W. E. McEwen, *ibid.*, **78**, 3063 (1956).

(3) (a) R. M. Caven, *J. Chem. Soc.*, **81**, 1362 (1902); (b) B. D. W. Luff and F. S. Kipping, *ibid.*, **95**, 1993 (1909); (c) F. S. Kipping and F. Challenger, *ibid.*, **99**, 626 (1911); (d) F. Ephraim, *Ber.*, **44**, 631 (1911); (e) W. J. Pope and C. S. Gibson, *J. Chem. Soc.*, **101**, 740 (1912); (f) A. E. Arbuzov and I. A. Arbuzova, *J. Russ. Phys.-Chem. Soc.*, **61**, 1905 (1929); *C. A.*, **24**, 5289[6] (1930).

(4) A. E. Arbuzov and G. Kh. Kamai, *J. Russ. Phys.-Chem. Soc.*, **61**, 2037 (1929); *C. A.*, **24**, 5736[4] (1930).

(5) H. S. Aaron and J. I. Miller, THIS JOURNAL, **78**, 3538 (1956).

(6) Recommendations of the Advisory Committee on the Nomenclature of Organic Phosphorus Compounds (*Chem. Eng. News*, **30**, 4515 (1952)) would give the name O-ethyl O-hydrogen ethylphosphonothionate to a compound of structural formula V. The fact that V is a fairly strong acid (*p*Ka *ca.* 2.3) is not readily apparent from this nomenclature. We use the O-ethyl ethylphosphonothioic acid designation, therefore, to emphasize the most important single physical characteristic of this compound. This name is slightly different from that originally[5] used; the new name is used to avoid designating the position of the proton in the free acid.

TABLE I

EFFECT OF CONCENTRATION AND OF ADDED ACID OR BASE ON THE SPECIFIC ROTATION[a] OF O-ETHYL ETHYLPHOSPHONOTHIOIC ACID (Va) IN AQUEOUS SOLUTIONS

Run	Concn. of acid Va, m./l.	Concn. of added HCl or NaOH, m./l.	$\alpha_{obsd.}$[b]	$[\alpha]_D$	$[\alpha]_D$ of Va anion
1	0.0345	0	-0.147 ± 0.006°[c]	-6.9 ± 0.3°	.....
2	.0340	0.0154 HCl	$-.113 \pm .006$[c]	$-5.4 \pm .3$	.....
3	.103	0	$-.064 \pm .008$	$-4.1 \pm .5$	.....
4	.265	0.123 HCl	$-.077 \pm .011$	$-1.9 \pm .3$	-10.7 ± 0.2°[d]
5	.500	0	$-.070 \pm .015$	$-0.9 \pm .2$	.....
6	.332	0.337 HCl	$+.033 \pm .009$	$+0.6 \pm .2$	-10.5 ± 0.3[e]
7	.276	1.01 HCl	$+.160 \pm .007$[f]	$+1.9 \pm .1$	.....
8	.0735	4.7 HCl	$+.180 \pm .006$[e]	$+4.0 \pm .1$	.....
9	.150	6 HCl	$+.204 \pm .008$[f]	$+4.4 \pm .2$	.....
10	.146	8 HCl	$+.195 \pm .010$[f]	$+4.3 \pm .2$	.....
11	.189	12 HCl	$-.077 \pm .007$	$-2.6 \pm .2$	-10.8 ± 0.7
12	.240	0.240 NaOH	$-.399 \pm .007$	.....	$-10.8 \pm .2$
13	.048	.148 NaOH	$-.159 \pm .006$[f]	.....	$-10.8 \pm .2$
14	.048	.148 NaOH	$-.166 \pm .008$[f]	.....	$-11.2 \pm .5$[g]
15	.048	.148 NaOH	$-.170 \pm .007$[f]	.....	$-11.5 \pm .5$[h]

[a] At a room temperature of 27 ± 2°, using the sodium D line. [b] 1-dcm. tube unless otherwise indicated. [c] 4 dcm. [d] After 1 day as the Va acid in the HCl solution. [e] After 22 days as the Va acid in the HCl solution. [f] 2 dcm. [g] After 1 day as the anion in NaOH solution. [h] After 22 days as the anion in NaOH solution.

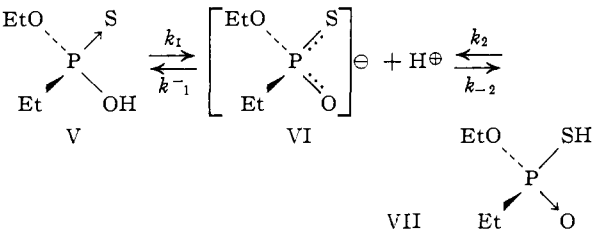
crystallized out of reagent grade acetone as monohydrate forms. The solubility difference between the quinine and the brucine systems has been used to advantage in one resolution of the acid. Here, the (−)-antipode Va was first obtained as the head crop from the quinine resolution. The more soluble and partially resolved tail crops were then converted to the brucine salt, and the (+)-antipode Vb now was obtained as the head crop from the new system.

The acids Va and Vb have been characterized both neat and as their dicyclohexylamine salts. The resolved acid may be distilled or dissolved in water in the presence of excess mineral acid or base without racemization. The very fact that the acid V could be resolved proved that the anion tends to maintain its (presumably) tetrahedral configuration, at least when paired with a cationic species in a relatively non-polar medium. The stability of the acid Va as demonstrated under the above described conditions, however, is proof of the fact that its configuration is markedly stable, and independent of the influence of an adjacent cationic center. Thus, the anion can be pictured as the resonance hybrid VI.

Some interesting polarimetric observations have been made with respect to the rotation of the acid Va in aqueous solutions, the results of which are summarized in Table I.

The observations recorded in Table I were made on Va, *i.e.*, that antipode of the acid which had a neat rotation of −13.6° (1 dcm.) and whose dicyclohexylamine salt had a specific rotation of −7.11° (methanol). As may be seen from Table I, the Va antipode also has a (−) rotation as its sodium salt in aqueous solution (*e.g.*, run 12), or as the free acid in dilute aqueous solution (*e.g.*, run 1). The specific rotation of the acid is affected, however, both by its own concentration and by the concentration of excess mineral acid. For example, by either increasing the concentration of the free acid Va or by adding hydrochloric acid, one obtains less negative values for the specific rotation, until finally a (+)-value is obtained which then levels off at *ca.* +4.4° in 5 to 8 *N* aqueous hydrochloric acid. In concentrated (12 *N*) hydrochloric acid, however, a value of −2.6° was obtained (run 11). That a reaction or a racemization had not occurred under these conditions was shown by the fact that the acid was converted to its sodium salt from both the concentrated hydrochloric acid solution (run 11) and the 0.337 *N* hydrochloric acid solution (run 6), which had stood for 22 days, with no apparent change in the specific rotation of the anion VI. The anion itself was shown to be stable in the presence of excess base (*p*H 13) for 22 days (run 15).

Some recent results have been interpreted[7] to indicate that the proton, in an acid of this type, tends to associate with the oxygen rather than the sulfur atom. This conclusion is in accord with what one would predict from a consideration of the relative basicities of these two atoms. However, since the ratio of the instantaneous concentration of the form with the proton on the oxygen atom to that with the proton on the sulfur atom has not been experimentally established, we have considered the observed rotation of the system to be a function of the concentrations of at least three species as represented by the equilibrium



Increasing the concentration of the acid V and VII or the addition of small quantities of a strong mineral acid will, of course, shift the above equilibrium. The addition of high concentrations of a

(7) M. I. Kabachnik, *et al.*, *Doklady Akad. Nauk, S.S.S.R.* **104**, 861 (1955); *C. A.*, **50**, 11240a (1956).

strong mineral acid, however, should affect the k-values, above, by affecting the ionization medium, and possibly even result in the formation of other (*e.g.*, protonated) asymmetric species.

It should be possible, at least in theory, to correlate the acid dissociation constant and the observed rotations of optically active O-ethyl ethylphosphonothioic acid in aqueous and aqueous acid solutions with the concentrations and specific rotations of the various optically active species present in the solution. We have been unable, as yet, to obtain a satisfactory correlation of this type by empirical methods, using the data presented in Table I. An assignment of the absolute value of the specific rotation of either form of the undissociated acid V and VII, therefore, cannot be made at this time. However, since the anionic form (VI) of the acid (*e.g.*, as its sodium salt) has a specific rotation which is independent of its own concentration or the presence of excess base, the optical purity of the acid is determined readily by means of its specific rotation in basic solution.

Experimental

The Resolution of O-Ethyl Ethylphosphonothioic Acid (V) with Quinine.—The acid V[8] (12 g., 0.078 mole) was added to a solution of 25 g. (0.077 mole) of anhydrous quinine in 300 ml. of hot reagent-grade acetone. Ether (300 ml.) was then added and the solution was cooled. After six hours, 15.9 g. (crop 1) of off-white prisms, m.p. 139–146°, was collected. Three recrystallizations of this head crop from acetone gave 8.9 g. of a monohydrate[9] of the quinine salt of (−)-O-ethyl ethylphosphonothioic acid (IXa·H_2O), hard off-white prisms, m.p. 151–153° (with dehydration), $[\alpha]^{26}D$ −96.6 ± 0.8° (α_{obsd} −1.990 ± 0.015°, acetone, 2 dm., c 1.030).

Anal. Calcd. for $C_{24}H_{35}O_4N_2PS \cdot H_2O$: C, 58.1; H, 7.1; neut. equiv., 496.6. Found: C, 58.5; H, 7.5; neut. equiv., 493, 490.

This material can be dehydrated (see below) to give the anhydrous salt IXa, m.p. 158–160°.

Ether (300 ml.) was added to the mother liquor of crop 1; after cooling, 7.0 g. of needles, m.p. 155–159°, was recovered and set aside. The filtrate was then concentrated to a volume of about 50 ml.; 200 ml. of ether was added, and a tail crop of 7.8 g., m.p. 158–164°, rapidly came down. Recrystallization of this tail crop from 50 ml. of acetone gave 5.2 g. of product, m.p. 163–166°. Another recrystallization, using 2.4 g. in 30 ml. of acetone, gave 1.1 g. of the quinine salt of (+)-O-ethyl ethylphosphonothioic acid (IXb), soft white needles, m.p. 166–168°, $[\alpha]^{25}D$ −81.7 ± 0.6° (α_{obsd} −1.613 ± 0.012°, acetone, 2 dm., c 0.9868). Further recrystallization did not change this melting point.

Anal. Calcd. for $C_{24}H_{35}O_4N_2PS$: C, 60.3; H, 7.4; neut. equiv., 478.6. Found: C, 60.0; H, 7.3; neut. equiv., 476, 474.

In subsequent resolutions, acetone alone was used as the solvent; nearly pure IXa·H_2O was thus obtained directly as the first crop. Occasionally, the two diastereoisomeric salts crystallized from middle fractions as a simple mixture of prisms (IXa·H_2O) and needles (IXb), both optically pure. The difference in the respective densities of these two salts was used to separate the mixtures. One such mixture (54 g.), for example, was stirred in low boiling petroleum ether, and the needles, which tended to float, were decanted with the solvent. This process was repeated several times, until only a residue of the dense prisms remained. The needles crops thus obtained (44 g.) were combined and recrystallized from 400 ml. of acetone; fractions of 23 g., m.p. 165–167°, and 15 g., m.p. 164–166°, respectively, were obtained.

(8) Kindly furnished by Dr. F. W. Hoffmann and co-workers, who will describe the preparation of alkylphosphonothioic acids in a forthcoming paper.

(9) Reagent grade acetone evidently has sufficient water present to allow the head crop to crystallize as the preferred monohydrate salt.

Although IXa·H_2O and IXb did show a significant difference in their specific rotations when acetone was used as the solvent, little if any difference could be detected between the respective rotations when methanol or chloroform was used.

Conversion of IXa·H_2O to IXa.—A sample of IXa·H_2O (2.3781 g.), m.p. 151–153°, which had been ground in a mortar, was dried to a constant weight by pumping under vacuum for five hours at 100°. The residue, which now melted at 158–162°, had lost 0.0924 g. This figure represents a 3.89% loss in weight (calculated for 1 mole of water: 3.63%). A sample of IXb showed no change in its melting point (166–168°) and lost no weight when dried under vacuum at 100° for 1 hour. Another sample of IXa·H_2O, m.p. 151–154°, was vacuum dried over phosphorus pentoxide at 100° for 3 hours to give IXa, m.p. 158–160°.

In another experiment, a sample of IXa·H_2O (0.43 g.) in 11 cc. of absolute ethanol was dehydrated by evaporating off the solvent. Petroleum ether and ether were added to the residue, and a crop of needles, 0.33 g., m.p. 157–159°, was obtained.

Anal. Calcd. for $C_{24}H_{35}O_4N_2PS$: C, 60.3; H, 7.4; neut. equiv., 478.6. Found: C, 60.4; H, 7.6; neut. equiv., 473, 465.

Recovery of (−)-*o*-Ethyl Ethylphosphonothioic Acid (Va) from its Quinine Salt.—The quinine salt (IXa·H_2O, 49.3 g.) was dissolved in 125 ml. of warm methanol; a solution of 4.5 g. of sodium hydroxide in 25 ml. of water was then added. The addition of water (600 ml.) precipitated the quinine as an amorphous solid. The aqueous solution was decanted, washed with two 200-ml. portions of chloroform, then 200 ml. of ether. The organic layers were combined and used to dissolve the precipitated quinine. The resulting solution was extracted with two portions of an aqueous solution of sodium hydroxide (3 g. in 300 ml.); the aqueous layers were combined and washed in turn with a little chloroform, then ether, and then added to the original aqueous phase. The combined aqueous solutions were acidified with 20 ml. of concentrated hydrochloric acid, then extracted with four 350-ml. portions of ether. The ether extracts were combined, dried over Drierite and filtered. The solvent was boiled off and the residue was distilled at 92–94° (1–2 mm.) to yield 12.9 g. of Va, $n^{25}D$ 1.4880, α_{obsd} −13.563 ± 0.006° (neat, 1 dm.). An additional yield of 0.3 g. of forerun, $n^{25}D$ 1.4870, and 0.4 g. of a final cut, $n^{25}D$ 1.4880, were also collected.

A sample of this acid which had $[\alpha]D$ −10.8 ± 0.2° (as the sodium salt: α_{obsd} −0.399 ± 0.007° in 5 ml. of water containing 1.20 ml. of 1.001 N sodium hydroxide, 1 dm., c 3.696) as obtained from a previous distillation had an equivalent weight of 157 (calcd. 154) and gave a dicyclohexylamine salt, m.p. 160–161°, in a 92% yield.

Recovery of (+)-O-Ethyl Ethylphosphonothioic Acid (Vb) from its Quinine Salt.—The quinine salt IXb (41.5 g. of combined tail crop fractions) was dissolved in 100 ml. of warm methanol and an excess of sodium hydroxide (4 g. in 25 ml.) was added. Chloroform (200 ml.) was then added and the mixture was shaken with 600 ml. of water. The layers were separated and worked up essentially as described under the recovery of Va from its quinine salts above. The product was distilled at 89–90° (1–1.5 mm.) to give Vb, α_{obsd} +13.205 ± 0.013° (neat, 1 dm.) in about a 70% yield.

The Resolution of O-Ethyl Ethylphosphonothioic Acid (V) with Brucine.—The acid V (46.2 g., 0.3 mole) was added to 0.3 mole of brucine in 2 liters of hot acetone. On cooling, 67 g. of salt was collected, then recrystallized six times from acetone–ether to give 17 g. of a diastereoisomeric head crop (VIIIa), m.p. 181–182°, $[\alpha]^{26}D$ −14.9 ± 0.2° (α_{obsd} −0.458 ± 0.007°, 1 dm., dimethylformamide, c 3.066). This salt (12.2 g.) was dissolved in 50 ml. of methanol, and 24 ml. of 1 N sodium hydroxide was added, followed by about 300 ml. of water. The brucine, which rapidly crystallized, was filtered off. The resulting solution was then worked up essentially as described above for the recovery of Va from its quinine salt. The residue thus obtained distilled at 77–79° (0.3 mm.) to yield 2.52 g. of (+)-O-ethyl ethylphosphonothioic acid (Vb), $n^{24.5}D$ 1.4885, $[\alpha]D$ +10.6 ± 0.2° (as the sodium salt: α_{obsd} +0.328 ± 0.007° in 5 ml. of water containing 1.3 ml. of 1.001 N sodium hydroxide, 1 dm., c 3.094). An additional 0.20 g., $n^{24.5}D$ 1.4875, was collected as a forerun.

In another resolution, 45 g. (0.1 mole) of combined, par-

tially resolved tail crops obtained from a quinine resolution of V were cut back to the free acid in the usual manner. The undistilled acid thus obtained was combined with anhydrous brucine (36 g.) in *ca.* 400 cc. of hot acetone. After standing overnight in the refrigerator, a crop of 25 g. was obtained, which, after one recrystallization from 300 cc. of acetone, gave VIIIa·H_2O, a monohydrate of the brucine salt of (+)-O-ethyl ethylphosphonothioic acid, 20 g., m.p. 163–165°.

Anal. Calcd. for $C_{27}H_{37}N_2O_6PS \cdot H_2O$: C, 57.4; H, 6.9; neut. equiv., 566.6. Found: C, 57.2; H, 6.9; neut. equiv., 568, 569.

When dried under vacuum over phosphorus pentoxide at 100° for three hours, a sample of the monohydrate, above, lost 3.36% of its weight (calcd. 3.18% for one mole of water) and gave the anhydrous salt, m.p. 181–182°; equivalent weight calcd. for $C_{27}H_{37}N_2O_6PS$, 548.6; found 553, 550, 548. Samples of both the m.p. 163–165° and 181–182° brucine salts were cut back to the free acid in the usual manner, then converted to dicyclohexylamine salts. The dicyclohexylamine derivative thus obtained (unrecrystallized) from the 180–181° salt (in 81% yield) had m.p. 160–161°, $[\alpha]_D$ +6.90 ± 0.25° (α_{obsd} +0.357 ± 0.013°, methanol, 1 dm., *c* 5.182); that obtained (unrecrystallized) from the 163–165° form (in 73% yield) had m.p. 160–161°, $[\alpha]_D$ +6.94 ± 0.22° (α_{obsd} +0.341 ± 0.011°, methanol, 1 dm., *c* 4.922).

Dicyclohexylamine Salt of *d,l*-O-Ethyl Ethylphosphonothioic Acid.—The acid V (0.80 g., 0.0052 mole) in 5 ml. of petroleum ether was added to a solution of 1.05 g. (0.0058 mole) of dicyclohexylamine in 5 ml. of petroleum ether. The product, which immediately crystallized, was filtered to give 1.60 g. (92%) of salt, m.p. 166–167.5°. Recrystallization of a 0.5-g. portion from acetone–petroleum ether gave 0.30 g., m.p. 166–168°.

Anal. Calcd. for $C_{16}H_{34}NO_3P$: C, 57.28; H, 10.22. Found: C, 57.2; H, 10.1.

Dicyclohexylamine Salts of Va and Vb.—The dicyclohexylamine salt prepared directly from the undistilled Va residue obtained from IXa·H_2O (m.p. 151–153°) as described above had m.p. 159–160.5°, $[\alpha]_D$ −7.11 ± 0.23° (α_{obsd} −0.153 ± 0.005°, methanol, 1 dm., *c* 2.150). A similar salt prepared from the residue obtained as above from a sample of IXb (m.p. 163–166°) had m.p. 158–160°, $[\alpha]^{25}_D$ +6.85 ± 0.25° (α_{obsd} +0.221 ± 0.008°, methanol, 1 dm., *c* 3.230).

Anal. Calcd. for $C_{16}H_{34}O_3NP$: C, 57.28; H, 10.22. Found: (−)-antipode, C, 57.38; H, 10.00; (+) antipode, C, 57.30; H, 10.02.

Determination of the Neutralization Equivalents of the Quinine and Brucine Salts.—The equivalent weights of the diastereoisomeric salts obtained from the resolutions were determined by titration of the salts (0.2 g.) in 25 ml. of 3:2 methanol:water with standard 0.1 *N* aqueous base. The change in the apparent *p*H was recorded *via* a *p*H meter and the end-point was obtained from a graphical plot of the titration data.

ARMY CHEMICAL CENTER, MD.

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGY, THE UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE AND DENTISTRY]

The Synthesis of 6-Ethyl-7-methyl-9-(1′-D-ribityl)-isoalloxazine and 6-Methyl-7-ethyl-9-(1′-D-ribityl)-isoalloxazine[1]

BY JOHN P. LAMBOOY

RECEIVED AUGUST 9, 1957

The unequivocal syntheses of these two flavins have been accomplished by the condensation of the appropriately substituted *o*-aminoazo compounds with barbituric acid. The same synthetic route has been employed for the synthesis of 6,7-diethyl-9-(1′-D-ribityl)-isoalloxazine while 6-ethyl-9-(1′-D-ribityl)-isoalloxazine has been prepared by an alternative procedure.

In 1936, Karrer and Quibell[2] reported the synthesis of riboflavin by the reduction of N-(D-ribityl)-2-phenylazo-4,5-dimethylaniline to the corresponding *o*-phenylenediamine which in turn was condensed with alloxan. It was subsequently suggested by the Merck group in the monumental paper by Tishler[3] and associates that the procedure employed by Karrer might have produced a mixture of two flavins, namely, riboflavin and isoriboflavin. They do not appear to have demonstrated this directly but the following observations lend strong support to their suggestion. Tishler and associates discovered that the preparation of the *o*-aminoazo compounds from 4,5-disubstituted ribitylanilines did not yield pure compounds but mixtures resulting from the introduction of the arylazo group into either the 2- or the 6-position. When these isomeric forms were separated and independently reduced to the *o*-phenylenediamines and then condensed with alloxan, the 2-arylazo isomer was converted to riboflavin while the 6-arylazo form became isoriboflavin.

In the same paper, Karrer and Quibell[2] reported the synthesis of 6-ethyl-7-methyl-9-(1′-D-ribityl)-isoalloxazine and simply stated that at the level of 10 micrograms per day it was nearly as potent as riboflavin in stimulating the growth of riboflavin deficient rats. The procedure used for the synthesis of this compound was the same as that for the synthesis of riboflavin, namely, the reduction of what might have been a mixture of *o*-aminoazo compounds to a mixture of *o*-phenylenediamines. Subsequent condensation of the reduction product with alloxan might have produced a mixture of the desired 6-ethyl-7-methyl-9-(1′-D-ribityl)-isoalloxazine and 5-methyl-6-ethyl-9-(1′-D-ribityl)-isoalloxazine.

An additional point of interest to us was that we had found that intermediate levels of 6,7-diethyl-9-(1′-D-ribityl)-isoalloxazine stimulated the growth of the riboflavin deficient rat.[4] This material could not, however, be considered an adequate substitute for riboflavin in the nutrition of the rat because the animals failed to survive. This raises

(1) This investigation was supported in part by research grant No. CY-2940 from the National Cancer Institute, United States Public Health Service.

(2) P. Karrer and T. H. Quibell, *Helv. Chim. Acta*, **19**, 1034 (1936).

(3) M. Tishler, K. Pfister, R. D. Babson, K. Ladenburg and A. J. Fleming, THIS JOURNAL, **69**, 1487 (1947).

(4) J. P. Lambooy and H. V. Aposhian, *J. Nutrition*, **47**, 539 (1952).