Diisopropyl Bromomethylphosphonate (XVII).—Fifty-three grams (0.16 mole) of silver salt XVI was suspended in 300 ml. of carbon tetrachloride dried over phosphorus pentoxide. A solution of 25.7 g. (0.16 mole) of bromine, dried over sulfuric acid, in 50 ml. of carbon tetrachloride was slowly added. A vigorous reaction ensued accompanied by the evolution of carbon dioxide. When the addition of the bromine was completed and the evolution of the carbon dioxide had ceased, the yellow silver bromide was removed by filtration. The carbon tetrachloride was distilled *in vacuo* to yield 41 g. of an oil which was dissolved in ether and washed with saturated sodium bicarbonate solution. A yield of 8 g. of crude product was obtained on evaporation of the ether. Distillation at 0.5 mm. yielded 3.4 g., b.p. 80–92°, $n^{25}D$ 1.4652, and 2.8 g., b.p. 92–104°, $n^{25}D$ 1.4733. The infrared spectrum of the lower boiling fraction was very similar to that of XVIII.

Diisopropyl Iodomethylphosphonate (XVIII). Method A.—A solution of 6.2 g. of bromide XVII and 4.28 g. of sodium iodide in 35 ml. of acetone was heated under reflux for two hours. After this time, only 200 mg. of sodium bromide had precipitated. The acetone was distilled under vacuum and the residue dissolved in 40 ml. of 2-butanone. The mixture was heated under reflux for 17 hours. A quantitative yield of precipitated sodium bromide (2.48 g.) was obtained. The solvent was distilled *in vacuo*. The residue was dissolved in 50 ml. of ether and 3 ml. of water. The ether solution was washed with 3 ml. of dilute sodium thiosulfate solution, dried over sodium sulfate and evaporated. The residue was distilled at 0.5 mm. yielding 0.6 g., b.p. 84–90°, $n^{25}D$ 1.4718, and 1.4 g., b.p. 90–92°, $n^{25}D$ 1.4798. The infrared spectrum of the major fraction was identical with that of the known compound prepared in method B.

Method B.—A solution of 53.6 g. (0.2 mole) of methylene diiodide and 41.6 g. of triisopropyl phosphite was heated to 145° at which point an exothermic reaction occurred. When the vigorous reaction subsided the residue was distilled at 0.4 mm. The fraction, b.p. 76–100°, was collected and fractionated at 0.5 mm. to yield 28.7 g. (46.9%), b.p. 80–83°, $n^{25}D$ 1.4802.

Anal. Calcd. for $C_7H_{16}IO_3P$: C, 27.47; H, 5.27; I, 41.46; P, 10.12. Found: C, 27.36; H, 5.52; I, 40.86; P, 10.30.

Debromination of Diethyl Bromomethylphosphonate (XIX).—Eight grams of diethyl bromomethylphosphonate (XIX) in 100 ml. of ethanol containing 10 g. of 10% Pd–C and 1.0 g. of magnesium oxide was shaken in a Parr apparatus under 45-pound pressure of hydrogen. The theoretical quantity of hydrogen was absorbed within one hour. The reaction mixture was filtered and the solvent distilled. The infrared spectrum of the residual oil was identical with that of a known sample of XX.

Diethyl β,β-Dicarboethoxyethylphosphonate (XXII).—To a solution of 3.44 g. of sodium in 100 ml. of ethanol there was added 24 g. of diethyl malonate. After 5 minutes, 41.7 g. of diethyl iodomethylphosphonate (XXI) was added and the reaction mixture heated under reflux for 2 hours. Most of the solvent was distilled *in vacuo*. The residue was dissolved in ether–dilute hydrochloric acid and worked up in the usual manner. Distillation of the product at 0.15 mm. gave 13.4 g. (30.4%), of crude XXII, b.p. 142–146°, $n^{25}D$ 1.4378. This material was not further purified but analyzed and used in the next step.

Anal. Calcd. for $C_{12}H_{23}O_7P$: P, 9.98. Found: P, 8.26.

β-Carboxyethylphosphonic Acid (XXIII).—One gram of the oily ester XXII was refluxed with 8 ml. of 48% hydrobromic acid for 6 hours. The acid was distilled under vacuum to yield a crystalline residue. This solid on recrystallization from acetone gave 50 mg. of XXIII, m.p. 149–156°. Recrystallization raised the m.p. to 166–167° (Arbuzov and Dunin[7] report a m.p. of 167–168°).

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

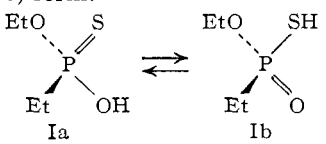
The Stereochemistry of Asymmetric Phosphorus Compounds. III. The Resolution of a Series of O-Alkyl Alkylphosphonothioic Acids[1]

BY HERBERT S. AARON, JANET BRAUN, THOMAS M. SHRYNE, HAROLD F. FRACK, GARY E. SMITH, ROY T. UYEDA AND JACOB I. MILLER

RECEIVED APRIL 15, 1959

A series of five O-alkyl alkylphosphonothioic acids [(RO)R'P(S)OH] were resolved, and the enantiomorphs thus obtained were characterized as their dicyclohexylamine salts. All of the *l*-enantiomorphs of these acids apparently possess the same configuration.

The resolution of O-ethyl ethylphosphonothioic acid (I) has been described.[2] This acid exists as a thionate (Ia) form in tautomeric equilibrium with a thiolate (Ib) form.



Ia Ib

In this sense, therefore, the acid possesses two reactive functional groups attached directly to the phosphorus atom. Since a host of asymmetric organophosphorus compounds can be synthesized through the reactions of these two functional groups, resolved acids of this general class become extremely useful for the synthesis of other resolved organophosphorus compounds. The compounds thus obtained can be used, in turn, to study the stereochemistry of displacement reactions at the asymmetric phosphorus atom.

We now wish to report the resolution of the following O-alkyl alkylphosphonothioic acids: O-ethyl methylphosphonothioic acid (II), O-isopropyl methylphosphonothioic acid (III), O-ethyl isopropylphosphonothioic acid (IV), O-methyl ethylphosphonothioic acid (V) and O-methyl methylphosphonothioic acid (VI).

The acids were resolved as their quinine or brucine salts and characterized as their dicyclohexylamine salts. The physical constants and analytical data on both the alkaloid and dicyclohexylamine salts of the resolved acids are summarized in Table I. Similar data on the *d*, *dl*-dicyclohexylamine salts of IV and V are given in the Experimental section; those of II, III and VI have been reported previously.[3]

(1) Presented in part before the 133rd Meeting of the American Chemical Society, San Francisco, Calif., April, 1958.

(2) H. S. Aaron, T. M. Shryne and J. I. Miller, THIS JOURNAL, **80**, 107 (1958).

(3) F. W. Hoffmann, B. Kagan and J. H. Canfield *ibid.*, **81**, 148 (1959).

TABLE I

PHYSICAL PROPERTIES AND ANALYTICAL DATA

Acid	Antipode	Alkaloid[a] Salts: M.p., °C.	Crystalline form	Empirical formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Dicyclohexylamine salts: M.p., °C.	[α]D[b]	Empirical formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
II	−	195–197[c]	Needles[d]	$C_{23}H_{33}N_2O_4PS$	59.46	59.3	7.16	7.2	123–124	−7.2°	$C_{15}H_{32}NO_2PS$	56.04	56.2	10.03	10.0
	+	197–199[e]	Prisms	$C_{26}H_{35}N_2O_6PS$	58.41	58.2	6.60	6.9	123.5–124.5	+7.0	$C_{15}H_{32}NO_2PS$	...	...	...	..
III	−	198–201	Needles	$C_{24}H_{35}N_2O_4PS$	60.23	60.2	7.37	7.5	123–123.5	−7.6	$C_{16}H_{34}NO_2PS$	57.28	57.6	10.21	10.0
	+	177–179	Prisms	$C_{24}H_{35}N_2O_4PS$	60.23	60.2	7.37	7.5	123–123.5	+7.6	$C_{16}H_{34}NO_2PS$	57.28	57.3	10.21	9.8
IV	−	133–134	Prisms	$C_{25}H_{37}N_2O_4PS.H_2O$	58.80	58.7	7.70	7.8	147–148	−7.0	$C_{17}H_{36}NO_2PS$	58.41	58.4	10.38	10.2
	+	148–150	Needles	$C_{25}H_{37}N_2O_4PS$	60.95	61.1	7.57	7.6	147–149	+6.5	$C_{17}H_{36}NO_2PS$	...	..	...	..
V	−	175–176	Needles	$C_{23}H_{33}N_2O_4PS$	59.46	59.2	7.16	6.9	185	−7.0	$C_{15}H_{32}NO_2PS$	56.04	56.6	10.03	9.9
	+	190–191	Powder	$C_{23}H_{33}N_2O_4PS$	...	..	..	..	184–185	+4.1	$C_{15}H_{32}NO_2PS$	56.04	56.5	10.03	10.1
VI	−	198–199[c]	Prisms	$C_{22}H_{31}N_2O_4PS$	58.65	58.6	6.94	7.0	180[c]	−2.3	$C_{14}H_{30}NO_2PS$	54.69	54.6	9.84	9.9
	+	198–200[c]	Needles	$C_{22}H_{31}N_2O_4PS$	58.65	58.3	6.94	7.1	180[c]	+6.6	$C_{14}H_{30}NO_2PS$	54.69	54.6	9.84	9.7

[a] Quinine salts unless otherwise indicated. [b] In methanol solution at 26–28°. [c] Melting points several degrees higher or lower generally could be obtained, depending upon the rate of heating. [d] Occasionally prisms were also obtained which had the same melting point as the needles; a mixed melting point of the two crystalline forms was not depressed. [e] Brucine salt head crop.

Four O-alkyl alkylphosphonothioic acids (I, II, III and IV) have now been resolved into enantiomorphs which have given dicyclohexylamine salts of equal (or nearly equal, in the case of IV) and opposite rotations. Interestingly enough, all of the optically pure dicyclohexylamine salts thus obtained had nearly the same specific rotations. Therefore, although antipodes of equal and opposite rotation were not obtained for V and VI, the (−)-antipode of V, at least, is judged to have been obtained in an optically pure form, because its dicyclohexylamine salt (obtained from a quinine salt which had been recrystallized to a constant melting point) had a specific rotation comparable to that of the known optically pure members of this series.

All of the *l*-enantiomorphs of these acids apparently possess the same configuration. This conclusion is based on the fact that optically active derivatives synthesized from the *l*-enantiomorphs of these acids (with the exception of V which was not similarly examined) were more potent inhibitors of cholinesterase enzymes than were their corresponding *d*-enantiomorphs.[4,5] In addition, the configurations of II and III have been correlated by a chemical method,[6] and the result is in agreement with that obtained from the biochemical method.

We have been using these acids to synthesize other optically active organophosphorus compounds, which in turn have been used to investigate the stereochemistry of substitution reactions at the asymmetric phosphorus atom. Detailed reports of this work will be published at a later date.

Experimental

Of the O-alkyl alkylphosphonothioic acids used in this work, samples of II and III[3] were kindly furnished by Dr. F. W. Hoffmann and co-workers of these laboratories; IV and V were synthesized as described below; VI was synthesized as previously described.[3] The O,O-dimethyl methylphosphonothioate intermediate used in the preparation of VI was synthesized by Mr. L. Thomsen, formerly of these laboratories.

The elemental analyses were carried out by personnel of the Analytical Research Branch of these laboratories. For the analyses, the compounds were ignited in the presence of vanadium pentoxide to obtain complete combustion.[7]

O-Ethyl Isopropylphosphonothioic Acid (IV).[8]—Sodium (9.2 g., 0.40 mole) was dissolved in 500 ml. of absolute ethanol, and the resulting solution was saturated with hydrogen sulfide gas. To the solution of sodium hydrogen sulfide thus obtained, there was added ethyl isopropylphosphonochloridate[9] (34 g., 0.20 mole), dropwise and with stirring; the reaction temperature was not allowed to rise above 30° during the addition. The ethanol was stripped off to near dryness, water was added, and the solution was again stripped down to remove the residual alcohol. The residue was dissolved in *ca.* 400 ml. of water, washed with ether,

(4) H. S. Aaron, H. O. Michel, B. Witten and J. I. Miller, THIS JOURNAL, **80**, 456 (1958).

(5) H. S. Aaron, H. O. Michel, G. E. Smith and J. I. Miller, unpublished results.

(6) H. S. Aaron, R. T. Uyeda and H. F. Frack, article being prepared for publication.

(7) C. A. Rush, "Miscellaneous Improvements in Microanalytical Technique," *Bibliography of Scientific and Industrial Reports*, PB-96865 (Jan., 1949), obtainable from the Office of Technical Services, U. S. Dept. of Commerce.

(8) Prepared according to a method developed by Mr. C. E. Williamson of these laboratories.

(9) F. W. Hoffmann, T. C. Simmons and L. J. Glunz III, THIS JOURNAL, **79**, 3570 (1957).

then acidified with excess (*ca.* 80 ml.) of concentrated hydrochloric acid. The product was then extracted with ether and the ether solution was dried with Drierite, filtered, then concentrated. The residue was distilled under reduced pressure to give IV, 19 g. (57%), b.p. 82–85° (0.45–0.5 mm.), $n^{25}D$ 1.4826 to 1.4833, for the four fractions collected; neut. equiv. 170, 171 (theory 168.2).

The dicyclohexylamine salt of IV, m.p. 163–164°, was prepared from this distillate in petroleum ether and recrystallized from ether.

Anal. Calcd. for $C_{17}H_{36}NO_2PS$: C, 58.41; H, 10.38; N, 4.01. Found: C, 58.5; H, 10.5; N, 4.2.

O-Methyl Ethylphosphonothioic Acid (V).—O,O-Dimethyl ethylphosphonothioate[10] (30 g., 0.20 mole), $n^{25}D$ 1.4753 (reported 1.4722), was hydrolyzed by stirring with 165 ml. of 1 *N* base (0.165 mole, 85%) at *ca.* 45° for 24 hours. About 4 ml. of unreacted starting material was still present as a second phase after this time. The reaction mixture was worked up as described[3]; the residue thus obtained was distilled to give V, 10 g., b.p. 66–70° (0.18 mm.), $n^{25}D$ 1.4976, neut. equiv. 145 (theory 140).

Anal. Calcd. for $C_3H_9O_2PS$: C, 25.68; H, 6.47. Found: C, 25.8; H, 6.4.

The dicyclohexylamine salt of V, m.p. 185°, was prepared in petroleum ether and recrystallized from acetone–ether.

Anal. Calcd. for $C_{15}H_{32}NO_2PS$: C, 56.04; H, 10.03; N, 4.36. Found: C, 55.8; H, 9.6; N, 4.4.

Resolutions.—The acids were resolved by recrystallization of their diastereoisomeric quinine (though in one case, brucine) salts from acetone solution until constant melting points were obtained. Because a variation in the melting points of these alkaloid salts could generally be obtained by varying the rate of heating, the melting point of a recrystallized crop was customarily determined simultaneously with that of the previously recrystallized crop in order to determine whether a change had occurred. The acids were liberated from their alkaloid salts as previously described,[2] except that the aqueous solutions of the acids were saturated with sodium chloride before extraction with ether. Except as indicated, the free acids were not isolated, but were converted into their dicyclohexylamine salts for characterization. The latter were obtained by distilling off the ether from the O-alkyl alkylphosphonothioic acid solution, taking up the residue in petroleum ether, and adding a slight excess of dicyclohexylamine. Occasionally, the amine was added before the distillation of the ether. The dicyclohexylamine salts thus obtained were recrystallized from ether or acetone–ether.

The (−)-antipodes of these acids, with the exception of VI, were obtained apparently optically pure from the corresponding quinine salt head crops. Of the levorotatory acids, only that of III was isolated as the free acid: b.p. 74–81° (0.3–0.5 mm.), $n^{25}D$ 1.4801 to 1.4806 (range of four fractions collected), α_{obsd} −13.92 ± 0.02° (neat, 1 dm.).

Of the dextrorotatory acids in this series, that of II was obtained from the head crop of its brucine salt, those of III, IV and V from the tail crops, VI from the head crop, of their respective quinine salts, all from acetone solution. In this manner, the (+)-antipodes of II and of III were obtained apparently optically pure; that of IV in a nearly (*ca.* 93%) optically pure state; that of V in an approximately 52% optically pure state. In this series, only the (+)-antipodes of II and III were isolated as the free acids: (+)-II, b.p. 79–84° (0.9–1.1 mm.), $n^{25}D$ 1.4920, $\alpha^{28.5}_{obsd}$ +9.57° (neat, 1 dm.); (+)-III, b.p. 59–64° (0.22–0.25 mm.), $n^{25}D$ 1.4802, α^{30}_{obsd} +13.59 ± 0.01° (neat, 1 dm.). Unlike as in the cases of I and II, the (+)-IV and (+)-V acids could not be conveniently obtained by resolution as a brucine salt from acetone solution. With IV, the diastereoisomeric brucine salts were too similar in their acetone solubilities to yield an effective separation, while with V, brucine formed a more insoluble salt with the (−)-antipode.

The resolution of VI was unique in two respects. First, both quinine and brucine formed a more insoluble salt with the *dextro* enantiomorph in acetone solution. Secondly, the head and the tail crop quinine salts, though of different crystalline character, had the same melting and mixed melting points, and their specific rotations were very similar. Therefore, neither the melting points nor the specific rotations of these quinine salts could be used to follow the extent of the separation of the head and tail crops in this system. The resolution was followed, however, by converting the various fractions to dicyclohexylamine salts for determination of their specific rotations. The highest specific rotation of a dicyclohexylamine salt obtained from this system was +6.6°. This derivative was prepared from a quinine salt obtained after seven recrystallizations of the original head crop. That this rotation represents the maximum obtainable for resolved VI is uncertain, since insufficient quinine salt remained for an eighth recrystallization. No attempt was made to carry the tail crop through to the same state of optical purity as was attained here for the head crop. Attempts to resolve VI with brucine in acetone or benzene–ether solutions, though not as carefully investigated, led to the same experimental difficulties.

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(10) F. W. Hoffmann, D. H. Wadsworth and H. D. Weiss, THIS JOURNAL, **80**, 3945 (1958).

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, THE HEBREW UNIVERSITY—HADASSAH MEDICAL SCHOOL, JERUSALEM, ISRAEL, AND THE DEPARTMENT OF MEDICAL CHEMISTRY, AUSTRALIAN NATIONAL UNIVERSITY, CANBERRA]

The Action of Mammalian Xanthine Oxidase on N-Methylated Purines

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RECEIVED JUNE 9, 1959

N-Methylation may alter either the rate or the course of the enzymatic oxidation of purines. 2-Hydroxypurine and its 1-methyl derivative are both attacked at carbon atom 8, while the 3-methyl compound gives 3-methylxanthine. 8-Hydroxypurine and its 7-methyl derivative are oxidized in position 2, while the 9-methyl isomer is refractory. These and other, related observations on N-methylated mono- and dihydroxypurines lead to an explanation of the exclusive pathway of oxidation of hypoxanthine *via* xanthine to uric acid. A new hypothesis on the mode of combination of xanthine oxidase with purine substrates has been based on the assumption that the enzyme binds a specific mesomeric or tautomeric form of the substrate, regardless of whether this form represents the major structure, present in solution.

In a recent study on the specificity of mammalian xanthine oxidase (XO),[2] a hypothetical mechanism for the oxidation of purines was derived from the behavior of hydroxypurines and certain of their N-methyl derivatives. It was assumed that purines become adsorbed onto the isoalloxazine ring of the prosthetic group in such a way that direct transfer of two hydrogens from the system $HN^{7}—C^{3}=C^{4}—C^{5}$ (as, *e.g.*, in the hydrated form of hypoxanthine [I]) to the corresponding positions N-1 and N-10 in the flavin moiety can take place. This hypothesis required that—when necessary—a hydrogen atom be placed on N-3 of the substrate by a hydration step, prior to the hydrogen transfer.

(1) Part of Ph.D. theses, submitted to the Faculty of Science, The Hebrew University, Jerusalem, Israel, 1960.

(2) F. Bergmann and S. Dikstein, *J. Biol. Chem.*, **223**, 765 (1956).