[DEPARTMENT OF EXPERIMENTAL THERAPEUTICS, LABORATORIO CUP]

Extraction of Reserpine and Other Alkaloids from Colombian *Rauwolfia hirsuta*

BY BERNARDO URIBE VERGARA

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Methods for the extraction and separation of reserpine, rauwolscine and alstonine from Colombian *Rauwolfia hirsuta* are described.

Rauwolfia hirsuta Jacq. or *Rauwolfia canescens* grows on the Atlantic coast and other regions of Colombia. Our preliminary work on that plant already has been published.[1,2]

The present paper describes the extraction from it of several alkaloids, the pharmacological properties of which have been studied by K. Mezey, and will be published elsewhere.

For the extraction of reserpine from *R. hirsuta*, it was not found necessary to separate and purify previously the "oleoresins," as it was done to obtain the alkaloid from other species.[3-5]

Experimental

Two kilograms of the finely powdered root of *R. hirsuta* were extracted with methanol until the solvent was alkaloid free. The extract was concentrated *in vacuo* to 2 l., diluted with an equal volume of water, and extracted with several 2-l. portions of chloroform. The combined chloroform solutions were chromatographed on a column with 200 g. of aluminum oxide, and the chromatogram eluted with 2 l. of chloroform. The eluate was evaporated to dryness *in vacuo*, and the residue recrystallized from methanol to give reserpine (1 g.).

The water–methanol solution left from the extraction of reserpine was concentrated *in vacuo* to remove the methanol, made slightly alkaline with ammonia (10%), and extracted several times with 2-l. portions of ether. The combined ether solutions were extracted with 2% tartaric acid, the tartaric acid solution was made alkaline with ammonia (10%), and the precipitated alkaloids filtered. From the ammoniacal filtrate a new portion of alkaloids was extracted with ether. The precipitated alkaloids were mixed with those obtained from the ethereal solution, dried and stirred several times with chloroform. The chloroform insoluble residue was filtered, washed with chloroform, redissolved in 2% tartaric acid, reprecipitated with ammonia, filtered and recrystallized from acetone, to give a small portion of an alkaloid which, according to its melting point and chemical behavior, seems to be sarpagine.

The chloroform solution was evaporated to dryness *in vacuo*, and the residue recrystallized from benzene, to give rauwolscine (10 g.).

The aqueous phase left from the extraction of the last two alkaloids was then made strongly alkaline with sodium hydroxide solution (10%), and extracted several times with ether to which 10% methanol was added; the combined organic phases were extracted with 10% acetic acid, the acetic acid solution alkalinized with sodium hydroxide solution (10%), and the precipitate filtered and recrystallized from ethyl alcohol, to give alstonine (1 g.).

The alkaloids were identified by their melting points and those of their derivatives. Equivalent weights were determined for rauwolscine and alstonine. All the experimental values compared well with those given in the literature.[3-9]

(1) K. Mezey and B. Uribe, *Anales de la Sociedad de Biologia de Bogotá*, **6**, No. 3, 127 (1954).

(2) K. Mezey and B. Uribe, *J. Pharmacol. and Exp. Therap.*, **110**, 38 (1954).

(3) L. Dorfman, A. Furlenmeier and others, *Helv. Chim. Acta*, **37**, 59 (1954).

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BOGOTÁ, COLOMBIA

[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORIES, THE LUBRIZOL CORPORATION]

Aromatic Phosphinic Acids and Derivatives. I. Diphenylphosphinodithoic Acid and Its Derivatives

BY WM. A. HIGGINS, PAUL W. VOGEL AND W. G. CRAIG

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A modified Friedel–Crafts synthesis has been used for the preparation of diphenylphosphinodithioic acid. The acid was the precursor for the synthesis of diphenylphosphinothioic chloride and diphenylphosphinic chloride. Diphenylphosphinothioic acid was prepared by the reaction of the thioic chloride with sodium hydroxide and also by the reaction of the phosphinic chloride with sodium hydrosulfide.

No satisfactory method for the preparation of diphenylphosphinodithioic acid has been reported in the chemical literature. The method of Malatesta[1] employing the Grignard reagent and phosphorus pentasulfide is probably the best, but gave only a 24% yield.

Benzene reacts with phosphorus pentasulfide and anhydrous aluminum chloride to give the crude acid in 70–90% yields. When eight moles of aluminum chloride per mole of phosphorus pentasulfide (P_4S_{10}) are used, the best yields are obtained after about eight hours. When the aluminum chloride was decreased to four moles, the yields were halved.

Pure diphenylphosphinodithioic acid melted at 55–56°. The only melting point reported previously for this material was 25–30°.[1] Oxidation with dilute nitric acid formed diphenylphosphinic acid which melted at 190–192°. The acid obtained by Malatesta from a similar oxidation of his dithioic acid melted at 188–190°.

(1) L. Malatesta, *Gazz. chim. ital.*, **76**, 167 (1947).

Toluene and chlorobenzene also have been employed in the phosphorus pentasulfide reaction, but the products from both of these reactions were mixtures of isomers which have not as yet been separated.

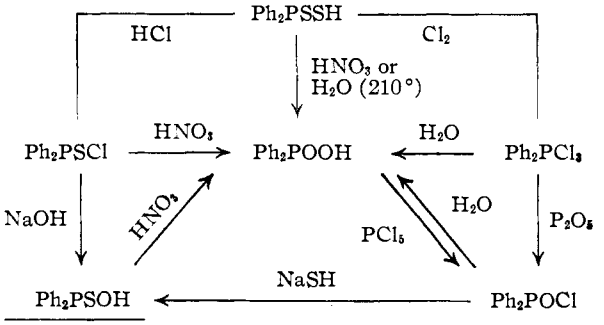
The conversion of diphenylphosphinodithioic acid to the thioic chloride by means of hydrogen chloride proceeds smoothly at 150–200° with elimination of hydrogen sulfide and formation of the thioic chloride in 70–80% yields. The method was found to be much more convenient and gave a better product than the conventional chlorination using phosphorus pentachloride which gave a 75% yield. Plets[2] reported the preparation of this material by sulfurization of diphenylphosphinous chloride. The boiling point reported by him, 275–280° at 15 mm., is higher than those observed in the present work, 200–210° at 4 mm. and 155–160° at 0.3 mm.

Diphenylphosphinothioic acid was prepared for the first time by hydrolysis of the thioic chloride in a warm sodium hydroxide solution. This product melted at 141–143°.

Diphenylphosphinic chloride was prepared in three ways: (a) diphenylphosphoranetrioic trichloride, prepared by the reaction of chlorine with diphenylphosphinodithioic acid, reacted with phosphorus pentoxide in benzene to give a 79% yield of the acid chloride; (b) diphenylphosphoranetrioic trichloride was heated with diphenylphosphinic acid in benzene at 70° to give a 73% yield of the phosphinic chloride; and (c) diphenylphosphinic acid in phosphoric trichloride was chlorinated with phosphorus pentachloride to give a 76% yield.

When diphenylphosphinic chloride was treated with anhydrous sodium hydrosulfide the product melted at 141–143°. A sample of this product mixed with diphenyl phosphinothioic acid obtained from diphenylphosphinothioic chloride showed no melting point depression.

Diphenylphosphinodithioic acid, the thioic chloride and the thioic acid all were converted to diphenylphosphinic acid by dilute nitric acid oxidation. An attempt was made to desulfurize the dithioic acid by means of steam. At 170° there was very little desulfurization, but when the acid was blown with superheated steam at 210° for two and one-half hours desulfurization was nearly complete, the resulting diphenylphosphinic acid containing only 0.54% sulfur.



(2) V. M. Plets, Dissertation, Kazan (G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 75).

Experimental[3]

Diphenylphosphinodithioic Acid.—A mixture of benzene (6630 g., 85 moles) and phosphorus pentasulfide (P_4S_{10}) (1887 g., 4.25 moles) at 50° was stirred rapidly and treated with anhydrous aluminum chloride (4540 g., 34 moles) over a period of three hours at such a rate that the temperature remained at 60–63°. The mixture was then refluxed (80–90°) for five hours, allowed to stand overnight, and poured onto crushed ice with vigorous stirring. Benzene was added and the solution was washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated, eventually at reduced pressure. The dark green, viscous liquid (3420 g., 80%) solidified on standing to a semi-crystalline mass melting at 30–50°.

The crude acid was dissolved in 10% sodium hydroxide solution, washed with benzene, acidified with hydrochloric acid, collected and dried under vacuum. It then was recrystallized twice from isopropyl alcohol to give white needles, m.p. 55–56° (over-all yield 54%).

Anal. Calcd. for $C_{12}H_{11}PS_2$: P, 12.38; S, 25.62; neut. equiv., 250. Found: P, 12.44; S, 25.92; neut. equiv., 249.

Diphenylphosphinothioic Chloride.—(a) A sample of crude diphenylphosphinodithioic acid was partially purified as indicated above, but not recrystallized. This acid (3000 g., 12 moles) was heated to 100° and treated with dry hydrogen chloride at a rapid rate over a period of three hours, during which time the temperature was gradually raised to 200°. The mixture was distilled and the pale yellow product, b.p. 155–160° at 0.3 mm., weighed 2305 g. (76%).

Anal. Calcd. for $C_{12}H_{10}ClPS$: Cl, 14.03; P, 12.26; S, 12.68. Found: Cl, 14.04; P, 12.22; S, 12.94.

(b) The chlorination of 1000 g. (4 moles) of diphenylphosphinodithioic acid by 834 g. (4 moles) of phosphorus pentachloride gave a 75% yield of the thioic chloride, b.p. 200–210° at 4 mm.

Anal. Calcd. for $C_{12}H_{10}ClPS$: Cl, 12.03; P, 12.26; S, 12.68. Found: Cl, 14.08; P, 11.81; S, 12.11.

Diphenylphosphoranetrioic Trichloride.—A vigorous stream of chlorine was passed into a solution of 1000 g. (4 moles) of diphenylphosphinodithioic acid in 1300 g. of carbon tetrachloride for a period of two hours while the temperature was maintained at 20–30° by means of a cold water-bath. The precipitated solid was collected under nitrogen and washed with cold carbon tetrachloride. When dried, the product weighed 1140 g. (98%). Because of its high reactivity with water vapor, no further attempt was made to purify it.

Diphenylphosphinothioic Acid.—(a) When diphenylphosphinothioic chloride was added dropwise to a 15% aqueous sodium hydroxide solution at 70°, the mixture acidified with hydrochloric acid and the solid recrystallized from toluene, a 95% yield of diphenylphosphinothioic acid as white needles, m.p. 141–143°, was obtained.

Anal. Calcd. for $C_{12}H_{11}OPS$: P, 13.23; S, 13.69. Found: P, 13.05; S, 13.45.

(b) Diphenylphosphinic chloride (prepared below) was added dropwise to excess sodium hydrosulfide in absolute ethanol at 0–10°. After standing overnight, the mixture was acidified and the product was recrystallized twice from toluene to give a 67% yield of the pure acid, m.p. 141–143°.

Anal. Calcd. for $C_{12}H_{11}OPS$: P, 13.23; S, 13.69. Found: P, 13.17; S, 13.80.

A mixture of the above acids showed no melting point depression.

Diphenylphosphinic Chloride.—(a) To a slurry of 146 g. (0.5 mole) of diphenylphosphoranetrioic trichloride in 300 cc. of benzene was added 27 g. (0.19 mole) of phosphorus pentoxide. The mixture was stirred for three hours at 30–35° and then filtered under nitrogen to remove a small amount of solid material. The filtrate was fractionated through a 12-inch Vigreux column. The colorless product boiled at 138–139° at 0.15 mm. and weighed 94 g. (79%).

Anal. Calcd. for $C_{12}H_{10}ClOP$: Cl, 14.98; P, 13.08. Found: Cl, 14.80; P, 12.75.

(b) To a slurry of 73 g. (0.25 mole) of diphenylphosphoranetrioic trichloride in 300 ml. of benzene was added 57 g. (0.26 mole) of diphenylphosphinic acid. The tem-

(3) All melting points are uncorrected.

perature of the reaction mixture dropped to 15°. The mixture was stirred for one-half hour and heated to the reflux temperature for three hours. It was filtered under nitrogen and the filtrate was fractionated through a 12-inch Vigreux column. The colorless product boiled at 157–158° at 0.55 mm. and weighed 86 g. (73%).

Anal. Calcd. for $C_{12}H_{10}ClOP$: Cl, 14.98; P, 13.08. Found: Cl, 14.88; P, 12.58.

(c) Diphenylphosphinic acid (109 g., 0.5 mole) was dissolved in 154 g. of phosphoric trichloride. Over a period of 1.5 hours 104 g. (0.5 mole) of phosphorus pentachloride was added. The temperature of the reaction mixture rose to 45°. The mixture was heated to 70–80° for three hours and fractionated through a 12-inch Vigreux column. The product boiled at 135–136° at 0.07 mm. and weighed 90 g. (76%).

Anal. Calcd. for $C_{12}H_{10}ClOP$: Cl, 14.98; P, 13.08. Found: Cl, 15.20; P, 12.72.

Diphenylphosphinic Acid.—(a) Both diphenylphosphinic chloride and diphenylphosphoranetrioic trichloride reacted very rapidly with water (or water vapor) to give quantitative yields of the acid. It was recrystallized from ethanol to form white needles, m.p. 190–192°.

(b) Diphenylphosphinodithioic acid, diphenylphosphinothioic chloride and diphenylphosphinothioic acid all were oxidized in benzene solution with 6 *N* nitric acid to form diphenylphosphinic acid which, after recrystallization from ethanol, melted at 190–192°.

(c) Steam was passed through 550 g. (2.2 moles) of diphenylphosphinodithioic acid at 125° for three hours. Analysis indicated that no reaction had occurred. Steam blowing was continued using preheated steam at 170° for 1.5 hours. Again there was practically no reaction. The temperature was raised to 210° and steam blowing was continued for 2.5 hours. As the reaction mass cooled, it solidified. The solid was broken up, filtered, washed with water and dried. This crude product weighed 400 g. (83%).

Anal. Calcd. for $C_{12}H_{11}O_2P$: P, 14.2; neut. equiv., 218. Found: P, 14.0; S, 0.54; neut. equiv., 207.

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CLEVELAND 17, OHIO

[CONTRIBUTION FROM THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH]

Synthesis of *N*-Phosphorylated Derivatives of Amino Acids[1]

BY SI-OH LI[2] AND ROBERT E. EAKIN

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The preparation of *N*-phosphorylated amino acids and their derivatives has been carried out in order to study their physical, chemical and biological properties and their possible involvement as intermediates in the biosynthesis of proteins. Such compounds were prepared by hydrogenolysis of appropriate *N*-dibenzylphosphoryl derivatives of amino acid esters and amino acid amides. The *N*-dibenzylphosphoryl amino acid esters and amides were prepared by the action of dibenzylphosphoryl chloride upon the corresponding compound in the presence of triethylamine. *N*-Phosphorylglycine and *N*-phosphoryl-DL-phenylalanine were obtained in crystalline form by hydrogenolysis of the corresponding *N*-dibenzylphosphoryl amino acid benzyl esters. The action of dibenzylphosphoryl chloride upon other functional groups of amino acids has been demonstrated.

When this investigation was undertaken phosphorylated amino acids had been suggested as the activated intermediates required for peptide syntheses.[3,4] These compounds would be analogous to the phosphorylated sugars and fatty acid intermediates in carbohydrate and lipid biogenesis, and a demonstration of their participation in peptide synthesis would indicate a mechanism for the utilization of the energy of adenosine triphosphate in the formation of amide bonds. The reactions of amino acids phosphorylated chemically at the carboxyl group had been investigated,[5] but little information was available on compounds in which the α-amino groups of amino acids are phosphorylated. Hence the preparation of such compounds was undertaken in order that a study could be made of their chemical, physical and biological properties.

The direct phosphorylation of α-amino acids with phosphorus oxychloride has been reported previously on two occasions,[6,7] but the yields obtained were low, and well characterized compounds were not isolated.

The phosphorylation of α-amino acids by diaryl- and dialkylphosphoryl halides has been attempted in two laboratories. In the first case[8] diphenylphosphoryl chloride was used, but the products obtained were the diphenyl phosphoric acid salts of the amino acids rather than the desired diphenyl phosphoramides. In the second instance[9] diisopropylphosphoryl fluoride was tried, but without success.

The phosphorylation of α-amino acid esters by dialkyl- and diarylphosphoryl halides has been accomplished and these products can serve as intermediates for the preparation of *N*-phosphoryl amino acids providing methods can be devised for the cleavage of both the phosphoryl and carboxyl esters. Amino acid esters have been phosphorylated with diphenylphosphoryl chloride[10] and with dialkylphosphoryl chlorides.[9,11] In both cases the reactions took place without difficulty and well characterized products were obtained. By hydrogenolysis of *N*-diethyl- and *N*-diisopropylphosphorylglycine benzyl esters, Wagner-Jauregg, *et al.*,[11] obtained the corresponding *N*-dialkylphosphoryl-

(1) This communication is from part of a dissertation submitted to the Graduate School of the University of Texas in partial fulfillment of requirements for the Ph.D. degree, May, 1954.

(2) Rosalie B. Hite Predoctorate Fellow, The University of Texas.

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