removed by filtration. The filtrate was concentrated *in vacuo* to about 3 ml., cooled in an ice-bath, and filtered. The filtrate was evaporated to dryness *in vacuo*. To the oily residue was added 20 ml. of acetone, followed by 60 ml. of ether, and the solution was stored 8 hours at 0°. The product was collected by filtration. There was obtained 1.36 g. of colorless needles, which, upon two crystallizations from methanol:acetone:isopropyl ether, yielded 1.06 g. of analytically pure material.

Four of the eight isomers (LDL, DLD, LDD, DLL) failed to crystallize from acetone or from acetone:ether solution; instead semi-solid products were obtained (Table III).

Valylvalylvaline Tripeptide Isomers. D-Valyl-D-valyl-D-valine.—0.500 g. (1.36 mmoles) of D-valyl-D-valyl-D-valine methyl ester hydrochloride was suspended in 11 ml. of 0.5 *N* potassium hydroxide, stored 75 minutes at 37°, chilled, and washed with three 2-ml. portions of ethyl acetate. The *p*H of the clear aqueous solution was adjusted to 5.0 with dilute acetic acid and the resulting solution was concentrated *in vacuo* to about 2 ml., 25 ml. of ethanol was added and the crystallizing solution was stored overnight at 0°. The crystalline product was collected by filtration, and recrystallized from 5 ml. of water and 30 ml. of ethanol; 0.371 g. (76% yield) of fine colorless plates was isolated. Flame photometry revealed less than 0.1% potassium. The product was dried to constant weight at 1 mm. at 100°, over P_2O_5, then exposed to room humidity until constant weight was reached. The tripeptides were hygroscopic and moistures were determined each time samples were weighed for characterization. The tripeptide isomers LLL, DDL and LLD were obtained in the same manner.

The four tripeptide methyl ester hydrochlorides which failed to crystallize were saponified as above and yielded crystalline peptides (Table IV).

PASADENA 4, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CARNEGIE INSTITUTE OF TECHNOLOGY]

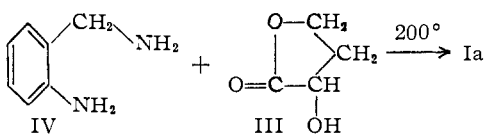
A New Synthesis of *dl*-Vasicine and a Methoxy Analog[1]

BY PHILIP L. SOUTHWICK AND JOSEPH CASANOVA, JR.[2]

RECEIVED SEPTEMBER 18, 1957

dl-Vasicine has been prepared from *o*-nitrotoluene by a reaction sequence in which *o*-nitrobenzylamine hydrochloride ethyl β-(*o*-nitrobenzylamino)-propionate hydrochloride, 1-(*o*-nitrobenzyl)-4-carbethoxy-2,3-dioxopyrrolidine, 1-(*o*-nitrobenzyl)-2,3-dioxopyrrolidine and 1-(*o*-nitrobenzyl)-3-hydroxy-2-oxopyrrolidine were the intermediates isolated and purified. A strictly parallel series of reactions, with only very slight modification of the details of procedure, served for the preparation of a new vasicine analog, *dl*-3-hydroxy-6-methoxypeg-9-ene ("*dl*-6-methoxyvasicine"), from 3-methyl-4-nitroanisole.

Work on the chemistry of vasicine (Ia) by several groups of workers[3] culminated in the synthesis of the alkaloid by Späth, Kuffner and Platzer[4] in 1935. The first synthesis depended on the preparation of methyl α-hydroxy-γ-aminobutyrate, which was treated with *o*-nitrobenzyl chloride to produce a low yield (*ca.* 7%) of 1-(*o*-nitrobenzyl)-3-hydroxy-2-oxopyrrolidine (IIa). Compound IIa, when treated with stannous chloride in hydrochloric acid, underwent reduction of the nitro group and spontaneous cyclization with dehydration to give a 35% yield of *dl*-vasicine. Later (1936) Späth and Platzer[5] described a more convenient procedure whereby α-hydroxybutyrolactone (III) was prepared from butyrolactone and heated to a high temperature with *o*-aminobenzylamine (IV) to give *dl*-vasicine, with a yield in the final step of 20%.



There have been indications in the literature[6] of interesting physiological effects, such as bronchodilator activity, produced by vasicine, and for this reason we have been prompted to undertake the synthesis of closely related analogs of vasicine in the hope of obtaining compounds of increased activity. In preparation for this effort we have investigated a new route to vasicine which proceeds through the final intermediate IIa of the first synthesis by Späth, Kuffner and Platzer,[4] but utilizes a different synthesis for this key compound. It was desired to achieve a preparative route which might be expected to be reliable when used on a relatively large scale and applicable without extensive modification when applied to the preparation of vasicine analogs It was also important that the synthetic sequence serve as an adequate structure proof for the products. These requirements led us to seek to avoid those cyclization methods used in the earlier syntheses which had produced low yields, necessitated the use of complex procedures for the isolation of the product, or utilized pairs of reactants whose manner of reacting might be considered uncertain.

The reaction sequence adopted is shown in Chart I. If vasicine analogs substituted in the aromatic ring were to be obtained by this route, a reasonably convenient method for the preparation of ring-substituted *o*-nitrobenzylamines (V) was needed. Chart II outlines a sequence which may prove to be applicable to the preparation of a number of such compounds. It was tested initially in the preparation of *o*-nitrobenzylamine itself (Va). *o*-Nitrotoluene was brominated with N-bromosuccinimide

(1) This investigation was supported in part by a research grant (RG-4371) from the Division of Research Grants, National Institutes of Health, Public Health Service.

(2) Institute Fellow in Organic Chemistry, 1956–1957. This paper is abstracted from a thesis submitted by Joseph Casanova, Jr., in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the Carnegie Institute of Technology, May, 1957.

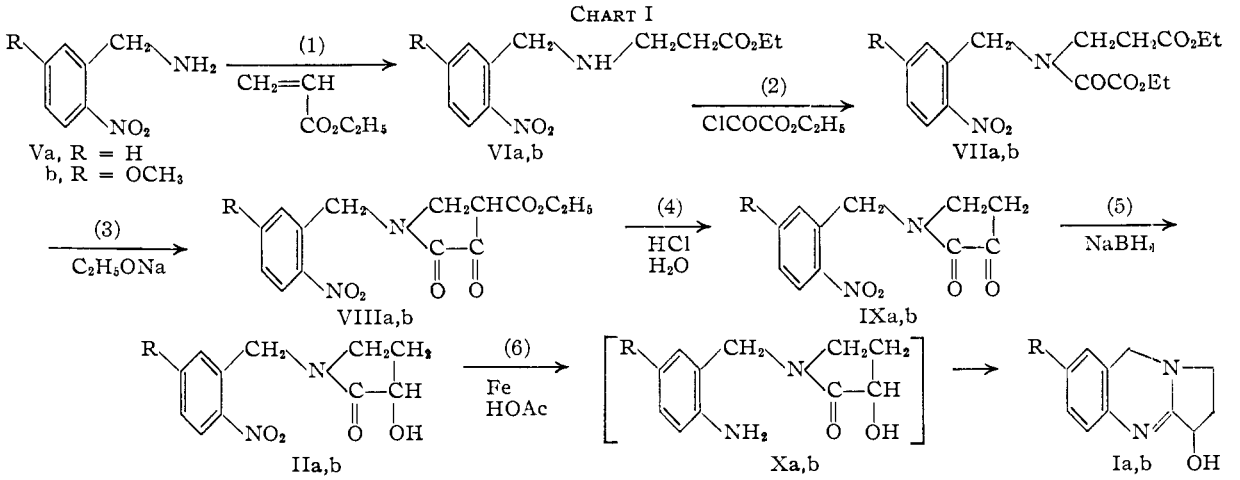
(3) Reviews of this work include those (a) by H. T. Oppenshaw (R. H. F. Manske and H. L. Holmes, "The Alkaloids," Vol. III, Academic Press, Inc., New York, N. Y., 1953, pp. 101–111); (b) by T. A. Henry, "The Plant Alkaloids," 3rd ed., P. Blakiston's Sons and Co., Inc., Philadelphia, Pa., 1939, pp. 544–548; and (c) by E. Späth, *Monatsh.*, **72**, 115 (1938).

(4) E. Späth, F. Kuffner and H. Platzer, *Ber.*, **68**, 699 (1935).

(5) E. Späth and N. Platzer, *ibid.*, **69**, 255 (1936).

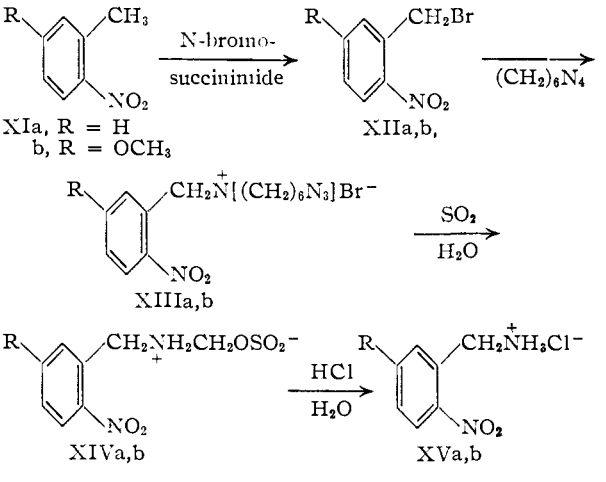
(6) (a) D. Hooper, *Pharm. J.*, [3] **18**, 841 (1888); (b) R. M. Chopra and S. Ghosh, *Indian Med. Gas.*, **60**, 354 (1925); *C. A.*, **19**, 3323 (1925).

CHART I



as described by Kornblum and Iffland,[7] and the resulting crude *o*-nitrobenzyl bromide (XIIa) was treated with hexamethylenetetramine to produce *o*-nitrobenzylhexaminium bromide (XIIIa).[8] This salt was then converted first into *o*-nitrobenzylaminomethylol sulfite (XIVa)[9] by treatment with water and sulfur dioxide, and thence into *o*-nitrobenzylamine hydrochloride (XVa) by hydrolysis of the methylol sulfite (XIVa) with hydrochloric acid. The conversion of XIa into XVa was only 19%, but the procedure proved to be reliable and suitable for use on a reasonably larger scale. The intermediate conversion of the hexaminium salt XIIIa to the methylol sulfite XIVa, as described by Reichert and Dornis,[9] provided an effective means of avoiding the loss of product through the formation of *o*-nitrobenzaldehyde.

CHART II



The conversion of *o*-nitrobenzylamine (Va) to the hydroxypyrrolidone IIa was accomplished largely by methods developed in the course of earlier investigations of the chemistry of pyrrolidine derivatives.[10] Thus, the first step (reaction 1, Chart I) was addition of *o*-nitrobenzylamine to ethyl acrylate to produce ethyl β-(*o*-nitrobenzylamino)-propionate (VIa) in a yield of 81%. This compound was purified and characterized in the form of the hydrochloride. However, the preparation of 1-(*o*-nitrobenzyl)-4-carbethoxy-2,3-dioxopyrrolidine (VIIIa) from intermediate VIa was not performed by direct condensation with ethyl oxalate,[10] as had been planned originally, for it was found that little or none of the desired dioxopyrrolidine VIIIa resulted from such a procedure. Undoubtedly the active hydrogen of the methylene group *ortho* to the nitro group in compound VIa diverted the condensation from its usual course. The difficulty finally was surmounted by treating the aminopropionate VIa with ethoxalyl chloride to form the N-ethoxalyl derivative VIIa, which then underwent the desired Dieckmann cyclization when treated with sodium ethoxide. A competing elimination reaction has sometimes interfered with such cyclizations in other cases,[10b] but the 4-carbethoxy-2,3-dioxopyrrolidine (VIIIa) was obtained from the aminopropionate VIa in 76% yield by this means.

The compound 1-(*o*-nitrobenzyl)-2,3-dioxopyrrolidine (IXa) was obtained by acid hydrolysis and decarboxylation of the 4-carbethoxy compound (VIIIa). This type of process, when conducted with similar compounds, has usually given yields of 65% or higher, although some variation of conditions has been necessary to suit the requirements of individual compounds. In the present instance yields tended to fluctuate in the 40–60% range when the reaction was conducted with 25% aqueous hydrochloric acid containing a small amount of ethanol. Experiments also were performed using a hydrobromic acid mixture prepared from a small amount of 48% hydrobromic acid dissolved in glacial acetic acid. This procedure, which, unlike the hydrochloric acid procedure, provided a homogeneous mixture throughout the reaction period, afforded a crude product of higher purity, but the yield was only 50%. Further investigation of reaction conditions for this step would be desirable.

The reduction of the carbonyl and nitro groups of the 2,3-dioxopyrrolidine IXa and the cyclization of the assumed intermediate Xa to vasicine

(7) N. Kornblum and D. C. Iffland, THIS JOURNAL, **71**, 2137 (1949).

(8) S. J. Angyal, P. J. Morris, J. P. Tetaz and J. C. Wilson, *J. Chem. Soc.*, 2141 (1950).

(9) B. Reichert and W. Dornis, *Arch. Pharm.*, **282**, 100 (1944); *C. A.*, **45**, 1969f (1951).

(10) (a) P. L. Southwick and L. L. Seivard, THIS JOURNAL, **71**, 2532 (1949); (b) P. L. Southwick and R. T. Crouch, *ibid.*, **75**, 3413 (1953); (c) P. L. Southwick, E. P. Previc, J. Casanova, Jr., and E. H. Carlson, *J. Org. Chem.*, **21**, 1087 (1956).

(Ia) was carried out as a two-step process. In the first step, the carbonyl group was reduced with sodium borohydride to give 1-(*o*-nitrobenzyl)-3-hydroxy-2-oxopyrrolidone (IIa). The yield of crude product from the reduction was about 80%, but losses in the course of purification reduced the yield of analytically pure material to 57%.

Späth, Kuffner and Platzer[4] obtained vasicine from compound IIa by reduction with stannous chloride in hydrochloric acid solution. Their yield for this conversion was only 35%, however, and we were unable to achieve significantly better results when we used the same reagents. However, the yield of vasicine from compound IIa was raised to 91% when the reduction was performed with iron and aqueous acetic acid. In both procedures the crude product isolated following treatment with the reducing agent was shown by examination of its infrared spectrum to be vasicine; the intermediate 1-(*o*-aminobenzyl)-3-hydroxy-2-oxopyrrolidone (Xa) evidently underwent cyclization rapidly and spontaneously to give the 3,4-dihydroquinazoline system found in the alkaloid, and this cyclization occurred prior to the sublimation which has been a customary step[4,5] in purification of synthetic vasicine.

The properties of the product from this synthesis coincide closely with those attributed to *dl*-vasicine in the literature. Thus the melting point (209–210° in vacuum) was close to that reported previously for synthetic *dl*-vasicine (211–212° in vacuum),[4,5] and the composition corresponded to that expected for vasicine. In the form of the free base the sample showed an ultraviolet maximum at 303 mμ (ε 8500), whereas the hydrochloride showed a maximum at 284 mμ (ε 3240). In the 5.5 to 6.5 μ region of the infrared the free base in chloroform solution showed bands at 6.10, 6.23 and 6.32 μ and the hydrochloride, measured in a Nujol mull, bands at 5.91, 6.16 and 6.33 μ.[11] These results are in accordance with those previously reported for vasicine by Witkop,[12] and the infrared spectrum of the free base does, in fact, coincide in detail with that of vasicine throughout the entire range from 2 to 12 μ.[13]

The attractive possibility of accomplishing reduction of the keto carbonyl and nitro groups of IXa in a single operation, has been explored using a platinum oxide (Adams) catalyst, but thus far without success. The failure of this kind of procedure to yield the desired results probably can be attributed to condensation of the rapidly formed aromatic amino group with an unreduced keto carbonyl group in another molecule. The ketonic carbonyl groups of 2,3-dioxopyrrolidines react readily with aromatic amines.[10c] If such reactions occurred here, resinous polymeric products such as were actually obtained might well be expected to form. The possibility of surmounting this difficulty has not been adequately explored.

(11) Infrared data were obtained with a Perkin–Elmer model 21 spectrophotometer, ultraviolet data with a Cary recording spectrophotometer. Solutions in 95% ethanol were used for the ultraviolet measurements on the free base, solutions in 0.1 *N* ethanolic hydrochloric acid for the measurements on the hydrochloride.

(12) B. Witkop, *Experientia*, **10**, 420 (1954).

(13) We are indebted to Dr. Bernhard Witkop for providing us with a copy of the infrared spectrum of vasicine.

The synthetic scheme was applied readily to the synthesis of an analog of vasicine (Ib) carrying a methoxyl group in the aromatic ring. If the nomenclature suggested by Späth[3c] for compounds in this series is used, this substance would be described as 3-hydroxy-6-methoxypeg-9-ene. It has, however, been convenient to identify this compound by the simple name 6-methoxyvasicine, and this name will be used in the following discussion.

The requisite starting material for the preparation of 6-methoxyvasicine, the compound 2-nitro-5-methoxybenzylamine (Vb), had not been described. Two methods of preparing this substance from 3-methyl-4-nitroanisole (XIb) were sufficiently investigated to assess their value. One route involved conversion of XIb into 2-nitro-5-methoxyphenylacetic acid by published procedures,[14] followed by Curtius degradation of the acid to yield Vb. The method proved to be laborious, however, because of the large number of steps involved, and the urethan intermediate was difficult to hydrolyze. Much better results were achieved by use of the type of reaction sequence used in the preparation of *o*-nitrobenzylamine (Va) from *o*-nitrotoluene (see Chart II). Bromination of compound XIb with N-bromosuccinimide yielded the expected benzyl bromide (XIIb) and this was converted to the hydrochloride of 2-nitro-5-methoxybenzylamine (XVb) *via* the hexaminium salt XIIIb and the methylol sulfite XIVb. The conversion of 3-methyl-4-nitroanisole achieved in this way was 32%, but much unchanged starting material was recovered, and the yield based on unrecovered starting material was 80%.

In the methoxy series the yields for the sequence described in Chart I were 99% for reaction 1, 76% over-all for reactions 2 and 3, 39% for reaction 4, 87% for reaction 5 and 59% for reaction 6. The over-all yield of 6-methoxyvasicine from compound Vb was 15%. This compares with an over-all yield of 16% in the preparation of *dl*-vasicine from *o*-nitrobenzylamine (Va).

dl-6-Methoxyvasicine showed spectroscopic characteristics much like those of *dl*-vasicine itself. Thus the free base showed an ultraviolet maximum at 300 mμ (ε 11, 650), whereas in ethanolic hydrochloric acid the band appeared at 292 mμ (ε 9,870). The infrared absorption resembled that of *dl*-vasicine closely in the 5.5 to 6.5 μ region, with bands at 6.09, 6.22 and 6.30 μ for the free base and 5.90, 6.16 and 6.30 μ for the hydrochloride (both measured as Nujol mulls).

Tests of the physiological activity of 6-methoxyvasicine are in progress, as is work on the synthesis of other vasicine analogs.

Experimental[15]

Preparation of *o*-Nitrobenzylamines (V).—*o*-Nitrobenzylamine (Va) and 2-nitro-5-methoxybenzylamine (Vb) were prepared in the form of their hydrochlorides (XVa and XVb) by the same reaction sequence, but the variation in experimental details was sufficient to require a separate description of the procedure used for each compound.

(14) C. F. Koelsch, THIS JOURNAL, **66**, 2019 (1944).

(15) Melting points are corrected. Microanalyses by Dr. G. Weiler and Dr. F. B. Strauss, Oxford, England, and Geller Laboratories, West Englewood, N. J.

o-Nitrobenzylamine Hydrochloride (XVa).—A stirred mixture of freshly distilled *o*-nitrotoluene (392 g., 2.85 moles), N-bromosuccinimide (392 g., 2.39 moles) and dibenzoyl peroxide (1.5 g.) in 600 ml. of carbon tetrachloride was refluxed for 18 hours on a steam-bath. The dark mixture was cooled and filtered to remove the succinimide. The carbon tetrachloride was distilled at reduced pressure and the residual oil was treated with 210 g. (1.50 moles) of hexamethylenetetramine in 750 ml. of chloroform. The mixture was then stirred and heated on a steam-bath for 3 hours to complete the reaction. After the mixture had been cooled, the solid product was collected by filtration, washed with 200 ml. of cold acetone, and air-dried to give 451 g. (53% based on N-bromosuccinimide, 44% based on *o*-nitrotoluene) of the hexaminium salt,[8] which was pale tan in color.

The hexaminium salt (50.5 g., 0.138 mole) was suspended in 300 ml. of cold water which had previously been saturated with sulfur dioxide. While sulfur dioxide was bubbled through at a slow rate, the mixture was stirred vigorously and cooled in an ice–salt-bath. The hexaminium salt dissolved slowly while the product precipitated from solution. A good deal of frothing accompanied the reaction. After 20 minutes gas bubbling was stopped, but stirring and cooling were continued for 1 hour longer. The product was filtered, washed with 100 ml. of cold water and air-dried. This product, *o*-nitrobenzylaminomethylol sulfite,[9] melted at about 135° (reported[9] m.p. 139.5°) and was sufficiently pure for further use. The yield was 28.7 g. (85%).

The methylol sulfite (24.6 g., 0.10 mole) was subjected to a rapid steam distillation in 40 ml. of 25% aqueous hydrochloric acid for 1 hour, while the volume was held constant. The resulting clear red solution was filtered hot and then cooled slowly. *o*-Nitrobenzylamine hydrochloride, m.p. 246–248° (reported[16] m.p. 248°) crystallized from the solution. The yield was 9.4 g. (50%). The compound can be purified by crystallization from 95% ethanol.

2-Nitro-5-methoxybenzylamine Hydrochloride (XVb).—To a well-stirred suspension of 167 g. (0.931 mole) of N-bromosuccinimide and 15 g. of dibenzoyl peroxide in 1 liter of Baker reagent grade carbon tetrachloride was added 172 g. (1.03 moles) of 3-methyl-4-nitroanisole (b.p. 120° (1 mm.)).[14] The mixture was stirred and refluxed for 7 hours on a steam-bath. It was cooled and filtered to remove succinimide, and the succinimide was washed with 150 ml. of cold ether. The combined filtrate and washings were decolorized by filtration through a 20 × 5 cm. column of chromatographic alumina (80–200 mesh). The column was washed with an additional 1.5 l. of ether and the combined solutions were evaporated at reduced pressure without the application of heat to yield 207 g. of a pale yellow oil. (Crystals, m.p. 112–114°, of what may be pure 2-nitro-5-methoxybenzyl bromide (XIIb) could be obtained from this oil by crystallization from carbon tetrachloride.)

The oil was dissolved in 750 ml. of chloroform and the solution was mixed with 70 g. (0.50 mole) of hexamethylenetetramine. The suspension was stirred and refluxed on a steam-bath for 2 hours while the insoluble salt precipitated. At the end of this time half of the chloroform was distilled and replaced by acetone. The hexaminium salt was separated by filtration of the chilled mixture and was washed with 100 ml. of cold acetone to remove a small amount of color. The product, which was nearly white and melted with decomposition at about 170°, weighed 116 g. This weight represents a conversion of 32% and a yield of 80.7%, since 110 g. of starting material was recovered.[17]

The 2-nitro-5-methoxybenzylhexaminium bromide (115 g., 0.298 mole) was added with vigorous stirring to 600 ml. of water which had been cooled below 5° and saturated with sulfur dioxide. A gentle stream of sulfur dioxide was continued for 10 minutes, and the mixture was stirred and cooled for 40 minutes longer. The white product, the methylol sulfite, was removed by filtration and washed with a small quantity of ice-water. It was pressed as dry as possible and then subjected to a rapid steam distillation in 120 ml. of 25% hydrochloric acid. The volume was maintained at 120 ml. throughout the reaction. After filtration, the solution was cooled below 5°, whereupon 2-nitro-5-methoxybenzylamine hydrochloride (XVb) separated as a tan crystalline solid. The solid was removed by filtration, pressed dry, and triturated with 100 ml. of acetone. After filtration and air-drying the weight of product was 64.1 g., which represents a quantitative conversion from the hexaminium bromide. The compound melted at 201.5–203.0° dec., and was sufficiently pure for use in further reactions.

The free amine Vb could be prepared as a solid by slow addition of sodium hydroxide solution to a cooled aqueous solution of the amine hydrochloride. After drying in a vacuum desiccator over Drierite and Ascarite it melted at 88–89°. The free base appeared to be light sensitive when impure and evidently reacted with atmospheric carbon dioxide when exposed to air. The amine was characterized by preparation of a phenylthiourea derivative, N-(2-nitro-5-methoxybenzyl)-N′-phenylthiourea.[18] Three crystallizations of the derivative from 95% ethanol gave pale yellow diamond-shaped plates, m.p. 158.5–159.0°.

Anal. Calcd. for $C_{15}H_{15}O_3N_3S$: C, 56.77; H, 4.76; N, 13.24. Found: C, 56.92; H, 4.83; N, 13.40.

Ethyl β-(*o*-Nitrobenzylamino)-propionate (VIa).—*o*-Nitrobenzylamine hydrochloride (20.4 g., 0.114 mole) was dissolved in a minimum volume of water and the solution was made strongly basic by addition of 10% sodium hydroxide. The clear oil which formed was extracted into two 120-ml. portions of ether. The ether was dried with 10 g. of anhydrous sodium sulfate, filtered, and finally dried over Drierite. The dried solution was filtered and evaporated, leaving a yellow oil. The oil was dissolved in 100 ml. of absolute ethanol and 11.4 g. (0.114 mole) of freshly distilled ethyl acrylate was added. This solution was allowed to stand overnight and the solvent was then removed by distillation from a steam-cone. The oily residue was dissolved in 200 ml. of dry ether, and the solution was cooled in an ice-bath, then treated with dry hydrogen chloride. When precipitation was complete the solid was filtered from the ether and dissolved in 120 ml. of boiling absolute ethanol. The hydrochloride crystallized from this solution on cooling to give 20.7 g. of the aminopropionate hydrochloride, m.p. 137.5–139.0°. By concentrating and cooling of the mother liquor an additional 5 g. was obtained, m.p. 136.5–138.5°, and by repeating this operation 1.1 g., m.p. 136–139°, was obtained, raising the total yield to 26.8 g. (81.4%). For analysis the hydrochloride was crystallized from absolute ethanol three times more, to give a melting point of 138.5–139.0°.

Anal. Calcd. for $C_{12}H_{17}O_4N_2Cl$: C, 49.91; H, 5.94; N, 9.70. Found: C, 49.91; H, 5.94; N, 9.51.

Ethyl β-(2-Nitro-5-methoxybenzylamino)-propionate (VIb).—2-Nitro-5-methoxybenzylamine (Vb) was liberated from the hydrochloride XVb in the manner described above for preparing the free amine Va from its hydrochloride, and the solid product, m.p. 88–89°, was washed with a small amount of cold water and dried in a vacuum desiccator over Drierite and Ascarite. Some loss of product was involved in this operation, as shown by a yield of 77% recorded in one experiment. The amine (46.6 g., 0.256 mole) was then dissolved in 100 ml. of absolute ethanol and 25 g. (0.25 mole) of freshly distilled ethyl acrylate was added. The solution was allowed to stand for 24 hours at room temperature, then was filtered through a 10 × 1.8 cm. column of chromatographic alumina to remove colored impurities. The column was washed with 150 ml. of absolute ethanol and the combined solutions were concentrated by evaporation under reduced pressure at a temperature of 40° or below. The crude product (70.9 g., 99%) was obtained as a rather dark oil, which was, however, suitable for use in the next step of the synthesis.

To prepare and characterize the hydrochloride, 0.8 g. of the crude product was dissolved in 10 ml. of ether and the solution was treated with dry hydrogen chloride until separation of the red, gummy precipitate was complete. After

(16) E. L. Holmes and C. K. Ingold, *J. Chem. Soc.*, **127**, 1811 (1925).

(17) The starting material was recovered from the filtrate obtained after the salt was removed from the reaction mixture. The solvents were removed at reduced pressure, then the residue was taken up in 500 ml. of ether. The ether solution was washed successively with 100 ml. of 10% ferrous ammonium sulfate, 100 ml. of 10% hydrochloric acid and 100 ml. of water, then dried over Drierite and filtered. After removal of the ether by distillation, the residual oil was distilled at 121° (1 mm.) to yield 110 g. of 3-methyl-4-nitroanisole.

(18) The procedure used was that of R. L. Shriner, R. C. Fuson and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th ed., John Wiley and Sons, Inc., New York, N. Y., 1956, p. 227.

removal of the ether by decantation the material was dissolved in a minimum volume of hot ethanol. Crystallization of the hydrochloride took place slowly over a period of several days when the solution was stored in the refrigerator. By filtration, followed by concentration and cooling of the mother liquors and a second filtration, two crops of crystals (total 0.6 g.) melting in the range 102–105° were obtained. After two recrystallizations from absolute ethanol the melting point was 106.5–107°.

Anal. Calcd. for $C_{13}H_{19}O_6N_2Cl$: C, 48.97; H, 6.01; N, 8.79. Found: C, 48.65; H, 6.05; N, 9.00.

1-(*o*-Nitrobenzyl)-4-carbethoxy-2,3-dioxopyrrolidine (VIIIa).—The hydrochloride of ethyl β-(*o*-nitrobenzylamino)-propionate (VIa) (14.4 g., 0.05 mole) was dissolved in a minimum volume of water and treated with a solution of 4 g. of sodium hydroxide in 25 ml. of water. The oil which formed was extracted into four 100-ml. portions of ether. The combined extracts were dried over anhydrous magnesium sulfate and then over Drierite. After filtration the solution was evaporated on a steam-bath. The residual oil was cooled and treated with 19.4 g. (0.15 mole) of ethoxalyl chloride[19] then heated on a steam-bath for 90 minutes. At the end of this time the clear red oil was cooled and added dropwise to a stirred, well-cooled solution of 6.9 g. (0.30 mole) of sodium in 85 ml. of absolute ethanol kept in an ice–salt-bath. The addition was carried out at such a rate that the temperature was maintained below 10°. The pale tan slurry which resulted was stirred for 30 minutes at room temperature and then poured into 300 ml. of hot water. The resulting clear yellow solution was cooled and stirred while concentrated hydrochloric acid (60 ml.) was added slowly. The white crystalline mass which precipitated was removed by filtration after being allowed to stand in an ice-bath for 0.5 hour to ensure complete precipitation. After filtration the product was washed with water and air-dried. It was crystallized from 400 ml. of 95% ethanol to yield 10.9 g. of white needles, m.p. 182–184° (red melt). Concentration and cooling of the mother liquor gave an additional 0.9 g. of white solid, m.p. 181–183° (red melt), raising the total yield to 11.8 g. (76.5%). The product gave an immediate red color with alcoholic ferric chloride solution. For analysis a sample was crystallized five times from 95% ethanol to give long white needles with a melting point of 183–184° (red melt).

Anal. Calcd. for $C_{14}H_{14}O_6N_2$: C, 54.90; H, 4.61; N, 9.15. Found: C, 54.60; H, 4.53; N, 9.17.

1-(2-Nitro-5-methoxybenzyl)-4-carbethoxy-2,3-dioxopyrrolidine (VIIIb).—The details of the procedure were essentially identical to those described immediately above for compound VIIIa. Crude ethyl β-(2-nitro-5-methoxybenzylamino)-propionate (VIb) (70.9 g., 0.25 mole) was ethoxalylated by heating with 68 g. (0.50 mole) of ethoxalyl chloride for 2 hours, then the derivative was cyclized by dropwise addition to a solution of 16 g. (0.7 mole) of sodium in 300 ml. of absolute ethanol held at 0–5°. Following crystallization from 95% ethanol a total of 64.2 g. (76%) of product was obtained in two crops of crystals melting in the range 165–168° and, after three recrystallizations from 80% aqueous ethanol, the fine, white needles melted at 167.5–168°.

Anal. Calcd. for $C_{15}H_{16}O_7N_2$: C, 53.57; H, 4.80; N, 8.33. Found: C, 53.28; H, 5.11; N, 8.60.

1-(*o*-Nitrobenzyl)-2,3-dioxopyrrolidine (IXa). **Procedure A.**—A mixture prepared from 15.4 g. (0.05 mole) of 1-(*o*-nitrobenzyl)-4-carbethoxy-2,3-dioxopyrrolidine (VIIIa), 15 ml. of 48% hydrobromic acid and 100 ml. of glacial acetic acid was refluxed for 65 minutes. The resulting dark mixture was poured onto 400 g. of ice. After the ice had melted, the suspension was filtered to remove a small quantity of solid and extracted with four 80-ml. portions of chloroform. The combined extracts were dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The product (5.88 g., 50%) was a pale yellow solid, m.p. 124–125°. The crude product was sufficiently pure for subsequent reactions. The dioxopyrrolidine could be crystallized with difficulty from benzene. Two such crystallizations did not change the melting point.

Anal. Calcd. for $C_{11}H_{10}O_4N_2$: C, 56.41; H, 4.30; N, 11.96. Found: C, 56.88; H, 4.31; N, 11.83.

(19) Prepared by a procedure described in ref. 10a. The b.p. was 129–130°.

The anil[10c] was prepared by heating 1 g. each of the dioxopyrrolidine and aniline in 15 ml. of absolute ethanol for ten minutes on a steam-bath. A solid precipitated almost immediately. A nearly quantitative yield of the anil was collected by filtering the cooled mixture. The melting point was 172–176° dec. Three crystallizations from absolute alcohol afforded thin yellow prisms, m.p. 178.5–179.0° dec.

Anal. Calcd. for $C_{17}H_{15}O_3N_3$: C, 66.01; H, 4.89; N, 13.59. Found: C, 66.12; H, 4.87; N, 13.73.

Procedure B.—1-(*o*-Nitrobenzyl)-4-carbethoxy-2,3-dioxopyrrolidine (VIIIa) (8.3 g., 0.027 mole) was suspended in a solution prepared from 300 ml. of 25% aqueous hydrochloric acid and 25 ml. of 95% ethanol. The mixture was refluxed and stirred for 100 minutes. Almost all of the suspended solid had dissolved at this time. The solution was cooled to 10°, filtered and extracted with four 75-ml. portions of chloroform. The combined extracts were dried over anhydrous magnesium sulfate and filtered. The product was obtained by evaporation of the solution at reduced pressure without the application of heat. The yield was 3.6 g. (57%) of a pale tan solid, m.p. 122–125°.

1-(2-Nitro-5-methoxybenzyl)-2,3-dioxopyrrolidine (IXb).—This compound was prepared from 1-(2-nitro-5-methoxybenzyl)-4-carbethoxy-2,3-dioxopyrrolidine (VIIIb) essentially by procedure B, as described in detail above for compound IXa. Hydrolysis of 15 g. (0.0447 mole) of VIIIb in a solution prepared from 500 ml. of 25% hydrochloric acid and 25 ml. of 95% ethanol was accomplished by refluxing for 80 minutes and extracting the product into chloroform. Concentration of the solution yielded a pale yellow solid which crystallized when it was dissolved in a small volume of chloroform and the solution was diluted with half its volume of low-boiling petroleum ether (b.p. 30–60°), then cooled overnight in a refrigerator. The yield obtained in this way was 4.62 g. (39%) of pale yellow crytals, m.p. 139–140°. Two recrystallizations from the chloroform–petroleum ether mixture gave pale yellow needles, m.p. 139–140.5°.

Anal. Calcd. for $C_{10}H_{10}O_5N_2$: C, 54.54; H, 4.58; N, 10.60. Found: C, 54.87; H, 4.52; N, 10.43.

The anil[10c] was prepared by dissolving 1.0 g. of the crude 2,3-dioxopyrrolidine in 20 ml. of 95% ethanol and adding 1.0 g. of aniline. After ten minutes of heating on a steam-bath the mixture was cooled and filtered to give 1.5 g. of a pale yellow solid, m.p. 176–177°. Four crystallizations from 95% ethanol yielded clusters of yellow prisms, m.p. 176.5–178.0°.

Anal. Calcd. for $C_{18}H_{17}O_4N_3$: C, 63.71; H, 5.05; N, 12.38. Found: C, 63.89; H, 5.06; N, 12.57.

1-(*o*-Nitrobenzyl)-3-hydroxy-2-oxopyrrolidine (IIa).—A solution of 6.00 g. (0.0256 mole) of 1-(*o*-nitrobenzyl)-2,3-dioxopyrrolidine (m.p. 124–125°) in 100 ml. of absolute ethanol was added in portions to a cooled suspension of 3.00 g. (0.081 mole) of 98% sodium borohydride in 25 ml. of chilled absolute ethanol. The mixture was allowed to stand for 24 hours at room temperature and then evaporated on a steam-bath under reduced pressure. The residual gelatinous material was treated with 100 ml. of 25% hydrochloric acid and the mixture was extracted with three 100-ml. portions of chloroform. The combined extracts were dried over anhydrous magnesium sulfate, filtered, and evaporated at reduced pressure. The residue was a pale yellow solid, 4.9 g., m.p. 128–140°. The product was purified by crystallization from absolute methanol followed by sublimation in high vacuum at 135° (1 μ), then another crystallization from absolute methanol. This purification yielded 3.44 g. (57.3%) of pale yellow cubic crystals, m.p. 147–148°. This was the highest melting point we were able to attain. Melting occurs without obvious signs of decomposition, and the m.p. is not changed by use of an evacuated capillary tube for the determination. The difference between our m.p. and that previously reported[4] (150–151° (vacuum)) would seem to be due to some difference in thermometer calibration or procedure in making the temperature reading.

Anal. Calcd. for $C_{11}H_{12}O_4N_2$: C, 55.93; H, 5.12; N, 11.86. Found: C, 56.28; H, 5.19; N, 12.10.

1-(2-Nitro-5-methoxybenzyl)-3-hydroxy-2-oxopyrrolidine (IIb).—Two grams (7.45 mmoles) of 1-(2-nitro-5-methoxybenzyl)-2,3-dioxopyrrolidine (m.p. 135–136°) was placed in 50 ml. of chilled absolute ethanol and 2.0 g. (54 mmoles)

of solid sodium borohydride (98%) was added. The mixture was allowed to stand at room temperature for 20 hours. Addition of 25 ml. of water and evaporation at reduced pressure left a gelatinous residue which was acidified with 25 ml. of 25% hydrochloric acid. The solution which resulted was extracted with one 50-ml. and three 30-ml. portions of chloroform. The extracts were combined and dried over anhydrous magnesium sulfate. Filtration of the drying agent and evaporation of the solvent at 40° under reduced pressure left 1.8 g. of a pale yellow solid which crystallized from absolute methanol to yield 1.71 g. (86.2%) of yellow cubic crystals, m.p. 145.5–147.0°. For analysis this material was crystallized twice more from absolute methanol to produce pale yellow cubes, m.p. 150–151°.

Anal. Calcd. for $C_{12}H_{14}O_5N_2$: C, 54.13; H, 5.30; N, 10.52. Found: C, 54.02; H, 5.53; N, 10.67.

dl-**Vasicine** (Ia).—A mixture of 0.740 g. (3.13 mmoles) of 1-(*o*-nitrobenzyl)-3-hydroxy-2-oxopyrrolidine (IIa) (m.p. 145.0–146.5°) and 1.50 g. of clean iron filings in 18 ml. of 50% aqueous acetic acid was heated on a steam-bath for one hour. After cooling in an ice-bath, the solution was made strongly basic with a 25% solution of sodium hydroxide and extracted for 12 hours with 50 ml. of ether in an apparatus for continuous extraction. During this time a white product separated from the ether. The suspension was cooled and the solid (m.p. 193–198° (dec., vacuum tube)) was collected by filtration. Sublimation in high vacuum at 150° (1 μ) gave 0.535 g. (91%) of *dl*-vasicine as a white solid, m.p. 202–208° (dec., vacuum tube). Crystallization from absolute methanol gave fine white needles, m.p. 209° (dec., vacuum).

For analysis the crude reaction product was sublimed in high vacuum, crystallized from methanol, then from water, sublimed in high vacuum again, and finally crystallized from methanol to give white crystals, m.p. 209–210° (dec., vacuum tube).

Anal. Calcd. for $C_{11}H_{12}ON_2$: C, 70.18; H, 6.43; N, 14.88. Found: C, 70.55; H, 6.53; N, 14.92, 14.90.

A hydrochloride was prepared by passing dry hydrogen chloride gas into a solution of 0.1 g. of vasicine in 25 ml. of absolute ethanol. The solvent was distilled to a small volume on a steam-bath and the solution was cooled. Addition of ether precipitated a white solid, which was filtered after the mixture had stood in the refrigerator for several hours. The dry white solid melted at 206–208° dec. The reported[20] melting point for this compound is 205–207° (in vacuum).

(20) E. Späth and F. Kuffner, *Ber.*, **67**, 868 (1934).

dl-**6-Methoxyvasicine** (Ib).—A mixture of 1-(2-nitro-5-methoxybenzyl)-3-hydroxy-2-oxopyrrolidine (IIb) (4.00 g., 15 mmoles, m.p. 147.5–148.5°) and clean iron filings (8.40 g., 150 mmoles) was treated with 100 ml. of 1:1 aqueous acetic acid. The mixture was stirred and heated on a steam-bath for 90 minutes. After cooling the mixture in an ice-bath, it was made strongly basic with a 25% solution of sodium hydroxide in water. The mixture, which contained a large amount of precipitated ferrous hydroxide, was extracted with two 200-ml. portions of chloroform, the layers being separated by centrifugation. The combined chloroform extracts were dried over anhydrous magnesium sulfate, filtered, and evaporated at 40° under reduced pressure. The buff colored residue which remained weighed 2.57 g. (79% yield).

The product was purified by high vacuum sublimation at 155° (1 μ), followed by a crystallization from 95% ethanol. 6-Methoxyvasicine (1.31 g.) crystallized as large thin plates, m.p. 223–224° (dec., vacuum). From the mother liquor of the crystallization was obtained an additional 0.63 g. of crystalline solid, m.p. 219–223° (dec., vacuum). The total yield of purified material was 1.98 g. (59%). This represents an over-all yield of 9.4% for the synthesis, based on 3-methyl-4-nitroanisole consumed. The over-all yield based on 2-nitro-5-methoxybenzylamine was 15%.

The product was prepared for analysis by a high vacuum sublimation, followed by two crystallizations from ethyl acetate, and a third crystallization from 95% ethanol. The melting point of material prepared in this manner was 223–224° (dec., vacuum).[21]

Anal. Calcd. for $C_{12}H_{14}O_2N_2$: C, 66.03; H, 6.47; N, 12.84. Found: C, 66.53, 65.45; H, 6.35, 6.54; N, 12.91.

The product retained a faint tan color which could be removed only by extensive sublimations and crystallizations. *dl*-6-Methoxyvasicine could be crystallized from water, ethanol, methanol, aqueous alcohol or ethyl acetate, with 95% ethanol giving the best results.

Acknowledgment.—The authors are indebted to Mr. Sheldon E. Cremer for technical assistance.

(21) For measurement of the infrared spectrum, the hydrochloride of *dl*-6-methoxyvasicine, m.p. 219–221°, was prepared by use of a procedure like that used for *dl*-vasicine hydrochloride.

PITTSBURGH 13, PENNA.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, YALE UNIVERSITY]

Imidazole Catalysis. III.[1] The Solvolysis of 4-(2′-Acetoxyphenyl)-imidazole

BY GASTON L. SCHMIR[2] AND THOMAS C. BRUICE

RECEIVED SEPTEMBER 9, 1957

The solvolysis of 4-(2′-acetoxyphenyl)-imidazole (I) has been found to occur with participation of the imidazolyl group. The solvolysis of I is compared to that of acetyl salicylate (participation of the carboxylate ion) and of *p*-acetoxybenzoic acid (no participation).

Introduction

The implication of a histidine residue in the catalytic activity of several hydrolytic enzymes[3] has led to studies of the reaction of imidazoles with various compounds susceptible to enzymatic hydrolysis (see ref. 1). The imidazole-catalyzed hydrolysis of phenyl acetates has been particularly well investigated[1,4,5] and provides a basis for further attempts to approximate the mode of action of hydrolytic enzymes through the use of model systems.

If, in the enzymatic hydrolysis of an ester, the decomposition of the enzyme–substrate complex is considered a solvolytic reaction involving participation of acidic and basic groups on the enzyme surface, then the study of the behavior of esters which incorporate an imidazolyl group in position

(1) For preceding paper in this series, see T. C. Bruice and G. L. Schmir, THIS JOURNAL, **80**, 148 (1957).

(2) Public Health Predoctoral Fellow of the National Institutes of Health, 1956–1957.

(3) For pertinent references, see ref. 4 and 5.

(4) M. L. Bender and B. W. Turnquest, THIS JOURNAL, **79**, 1652, 1656 (1957).

(5) T. C. Bruice and G. L. Schmir, *ibid.*, **79**, 1663 (1957).