sium iodide to make the solution approximately 1*M*, and 0.1*M* iodine was added drop by drop until a permanent iodine color remained. After the solution had stood for 10 min., 0.1*M* sodium thiosulfate was added drop by drop until the iodine color was just discharged.

Acknowledgment. The polarimetric measurements described in this paper were conducted largely in the Department of Biochemistry of the University of California at Berkeley; the kind hospitality afforded by that institution is gratefully acknowledged. Professor Joseph B. Neilands offered valuable advice and helpful discussion; Mr. Donald Sproul assembled and maintained the photoelectric polarimeter which was used. That portion of the work was performed under Contract No. AF 18(603)-135, with the United States Air Force, Office of Scientific Research and Development, Aeromedical Division. The remainder of this work was supported by Grant G-4669, United States Public Health Service.

STILLWATER, OKLA.

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

Synthesis of a Chloro Derivative of DL-Vasicine[1]

PHILIP L. SOUTHWICK AND SHELDON E. CREMER

Received December 3, 1958

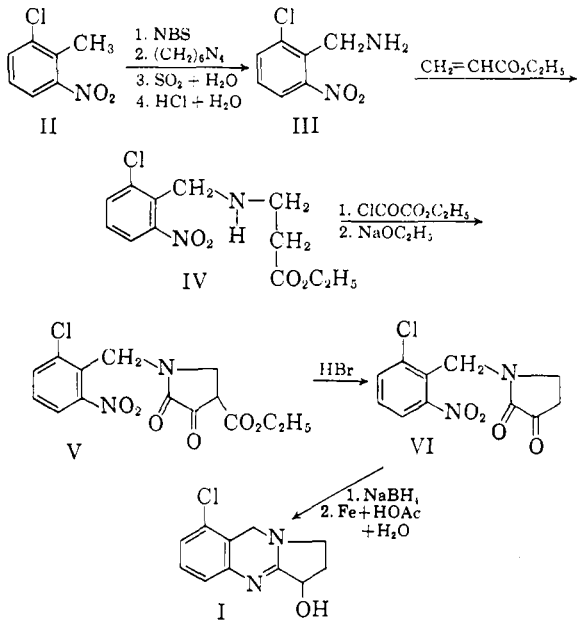
DL-7-Chlorovasicine (7-chloro-3-hydroxypeg-9-ene) has been prepared from 2-chloro-6-nitrotoluene. The compound displays a moderate activity against histamine-induced bronchospasm in guinea pigs, an activity which is potentiated by simultaneous administration of atropine. DL-Vasicine is less active, and DL-6-methoxyvasicine (6-methoxy-3-hydroxypeg-9-ene) showed no activity of this kind.

In a recent publication[2] from this laboratory there was described a new scheme of synthesis for the alkaloid vasicine which was successfully applied to the synthesis of an analog carrying a methoxyl group in the 6- position of the pegene ring system. The method has now been extended to the synthesis of a chloro derivative (I), which could be described as DL-7-chloro-3-hydroxypeg-9-ene according to the system of nomenclature introduced by Späth,[3] and which, for convenience, we have designated DL-7-chlorovasicine.

The general synthetic scheme was discussed in some detail in the previous paper.[2] In the present instance the starting point for the synthesis was the commercially available 2-chloro-6-nitrotoluene (II). The methyl group of II was brominated by use of *N*-bromosuccinimide (NBS),[4] and the crude bromide so obtained was converted into 2-chloro-6-nitrobenzylamine (III) in an over-all yield of 24.8% from II by first forming the hexaminium salt with hexamethylenetetramine and then hydrolyzing this product *via* an intermediate methylol sulfite.[2,5]

The 2-chloro-6-nitrobenzylamine (III) was treated with ethyl acrylate to produce a 93% yield of ethyl β-(2-chloro-6-nitrobenzylamino)-propionate, (IV), which was characterized in the form of the hydrochloride. Compound IV reacted with ethoxalyl chloride to from the *N*-ethoxalyl derivative, which, upon treatment with sodium ethoxide, underwent a cyclization of the Dieckmann type to yield 1-(2-chloro-6-nitrobenzyl)-4-carbethoxy-2,3-dioxopyrrolidine (V). The over-all yield of V from III was 44.6%.

Completion of the synthesis of DL-7-chlorovasicine (I) involved hydrolysis and decarboxylation of V to yield 1-(2-chloro-6-nitrobenzyl)-2,3-dioxopyrrolidine (VI) (74.5% yield), followed by two reduction steps. Reduction of crude VI with



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

(2) P. L. Southwick and J. Casanova, Jr., *J. Am. Chem. Soc.*, **80**, 1168 (1958).

(3) E. Späth, *Monatsh.*, **72**, 115 (1938).

(4) *Cf.* N. Kornblum and D. C. Iffland, *J. Am. Chem. Soc.*, **71**, 2137 (1949).

(5) *Cf.* B. Reichert and W. Dornis, *Arch. Pharm.*, **282**, 100 (1944); *Chem. Abstr.*, **45**, 1969 (1951).

sodium borohydride yielded 1-(2-chloro-6-nitrobenzyl)-3-hydroxy-2-oxopyrrolidine (VII), which was not purified, but was treated with iron and aqueous acetic acid to reduce the nitro group and permit spontaneous condensation of the resulting amino group with the lactam (pyrrolidone) carbonyl to yield I. The over-all yield of crude DL-7-chlorovasicine (I) from V was 50%, but purification losses reduced this figure to 16.8% of crystallized product. Thus the over-all yield of I from 2-chloro-6-nitrotoluene (II) was approximately 2%.

DL-7-Chlorovasicine has an infrared spectrum very similar to that of vasicine itself,[6] with prominent bands in the 5.5–6.5μ region at 6.10μ, 6.25μ, and 6.39μ (measured in chloroform solution). Comparison of this spectrum with that of vasicine[6] and 6-methoxyvasicine[2] shows that three bands (listed in decreasing order of intensity) at 6.09-6.10μ, 6.22–6.25μ, and 6.30–6.39μ are common to all three substances, and probably can be regarded as characteristic of simple vasicine derivatives.

In 1925 Chopra and Ghosh[7] reported that natural vasicine produced "a slight but a persistent broncho-dilation" which was rendered much more pronounced by simultaneous administration of atropine. These earlier results are supported in some degree by the finding that a sample of our synthetic DL-vasicine[2] had a slight activity against histamine-induced bronchospasm in guinea pigs, and that this activity was markedly potentiated by atropine. Toxic effects which resulted in the death of some animals were observed at dose levels as low as 5 mg./kg. when vasicine was administered intraperitoneally without atropine. DL-7-Chlorovasicine (I) proved to be more active (rated moderately active) and less toxic (no apparent side effects at 25 mg./kg.) than DL-vasicine itself when tested in guinea pigs in the same fashion. Again the activity was markedly potentiated by atropine. DL-Vasicine administered alone at intravenous dose levels up to 5 mg./kg. showed no bronchodilator activity in the pithed dog. DL-6-Methoxyvasicine appeared to be devoid of antihistaminic and bronchodilator activity.

EXPERIMENTAL[8]

2-Chloro-6-nitrobenzylamine (III). A mixture prepared from 202 g. (1.18 mole) of 2-chloro-6-nitrotoluene, 230 g. (1.29 mole) of *N*-bromosuccinimide, 10 g. of benzoyl peroxide, and 1 l. of carbon tetrachloride was refluxed on a steam bath for 14 hr. with vigorous stirring. The mixture was cooled for 1 hr. in an ice bath, then filtered to remove the succinimide, which was extracted with 200 ml. of ether. The ether extract was added to the chloroform filtrate, and the resulting solution was passed through a 5 × 55 cm. column of alumina (1 lb., 80–200 mesh). The column was then washed with 1.5 l. of ether, and the total eluate was concentrated by evaporation under reduced pressure at a temperature maintained at 40° or below. A pale yellow oil was obtained, weight 310 g.

The oil was added cautiously to 165 g. (1.18 moles) of hexamethylenetetramine in 800 ml. of chloroform. The reaction began promptly with the evolution of heat. (Cooling may at times be needed to prevent overheating of the reaction mixture.) After the initial reaction had subsided somewhat the mixture was refluxed and stirred for 2 hr., then was cooled in an ice bath for several hours before the hexaminium salt was collected by filtration. The filter cake was washed with small portions of cold acetone. The separation of a second crop of the salt was induced by concentrating the filtrate to about one half the original volume and diluting the solution with acetone. The crude hexaminium salt was partially purified and freed of colored impurities by trituration with warm absolute ethanol, followed by cooling of the mixture in an ice bath and removal of the product by filtration. The product (284 g.; 61.6% yield) was a white powder m.p. 174–178°.

For conversion of the hexaminium salt to the benzylamine *via* the methylol sulfite, 197 g. (0.504 mole) of the hexaminium salt was added rapidly with stirring to 700 ml. of water at 5° which had been saturated with sulfur dioxide. After 1 hr. of stirring with cooling and continuous addition of sulfur dioxide, the product (the amine methylol sulfite) was collected by filtration and dried. The white powder, m.p. 164–168° with previous softening, weighed 90.0 g. (63.7% yield).

The methylol sulfite (120 g., 0.427 mole) was added to 250 ml. of 25% hydrochloric acid. The mixture was steam distilled for 3 hr. The volume was maintained at *ca.* 250 ml. throughout the distillation by regulating the rate of introduction of steam and the heating of the distillation vessel. The solution was next cooled and made strongly basic by addition of sodium hydroxide. The mixture was extracted with three 300-ml. portions of ether and the combined ether extract was dried over Drierite. The mixture was filtered and the filtrate was concentrated under reduced pressure to yield 2-chloro-6-nitrobenzylamine (50.5 g.; 63.1% yield) in the form of a low-melting yellow solid. The over-all yield from 2-chloro-6-nitrotoluene was 24.8%.

The hydrochloride was prepared in order to characterize 2-chloro-6-nitrobenzylamine (III). The free amine (*ca.* 2.5 g.) was dissolved in dry ether and the hydrochloride was precipitated by addition of dry hydrogen chloride. The compound was obtained as pale yellow diamond-shaped plates, m.p. 258–260°, following three crystallizations from absolute ethanol.

Anal. Calcd. for $C_7H_8O_2N_2Cl_2$: C, 37.69; H, 3.62; N, 12.56. Found: C, 38.03; H, 3.64; N, 12.55.

Ethyl β-(2-chloro-6-nitrobenzylamino)-propionate hydrochloride (IV). To a mixture prepared from 48.0 g. (0.256 mole) of 2-chloro-6-nitrobenzylamine (III) and 150 ml. of absolute ethanol, 25.6 g. (0.256 mole) of freshly distilled ethyl acrylate was added. The mixture was allowed to stand for 24 hr., and the solvent was then removed by distillation under reduced pressure from a steam cone. The residual oil was dissolved in 300 ml. of dry ether and dry hydrogen chloride was added until precipitation was complete. The product was removed by filtration and dried in a vacuum desiccator. The yield was 77.0 g. (93%) of a white product, m.p. *ca.* 180°. Following two crystallizations from absolute ethanol and a final crystallization from acetone, white hexagonal prisms were obtained, m.p. 180–181.5°.

Anal. Calcd. for $C_{12}H_{16}O_4N_2Cl_2$: C, 44.59; H, 4.99; N, 8.67. Found: C, 44.30; H, 4.81; N, 8.83.

1-(2-Chloro-6-nitrobenzyl)-4-carbethoxy-2,3-dioxopyrrolidine (V). A solution prepared by dissol ing 100 g. (0.310 mole) of ethyl β-(2-chloro-6-nitrobenzylamino)propionate hydrochloride (IV) in a minimum volume of water was made basic by addition of an aqueous solution contairing 20 g. (0.5

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

(7) R. M. Chopra and S. Ghosh, *Indian Med. Gaz.*, **60**, 354 (1925).

(8) Melting points are corrected. The melting points of samples of 7-chlorovasicine were taken in evacuated capillaries. Microanalyses by Geller Microanalytical Laboratories, Bardonia, N. Y.

mole) of sodium hydroxide. The mixture was then extracted with three 300-ml. portions of ether, and the combined ether extract was dried over magnesium sulfate, filtered, and concentrated under reduced pressure at a temperature of about 40°. The residual oil was added dropwise to 46.5 g. (0.341 mole) of ethoxalyl chloride. The mixture was heated on a steam cone for 3.5 hr. in an apparatus protected from moisture.

The crude *N*-ethoxalyl derivative so obtained, a light orange viscous oil, was added dropwise over a 45-min. period to a vigorously stirred solution of 15.6 g. (0.68 mole) of sodium in 350 ml. of absolute ethanol which was held at a temperature of −5° in an apparatus protected from moisture. After a half hour of stirring without cooling the mixture was poured into 1 l. of boiling water. After the solution had cooled it was stirred and acidified by addition of *ca.* 1.2 moles of 6*N* hydrochloric acid. The mixture was kept overnight in a refrigerator, and the precipitated product was collected by filtration, then triturated with ether to remove colored impurities. The yield was 51.0 g. (48%) of a light tan product which was purified by crystallization from 95% ethanol to give 36 g. of light gray needles. (A second crop of 10 g. was recovered from the mother liquors.) A sample was purified by three crystallizations from absolute ethanol to give needles, m.p. 193–195.5° (dec., red melt) which retained a trace of gray color.

Anal. Calcd. for $C_{14}H_{13}O_6N_2Cl$: C, 49.35; H, 3.85; N, 8.22. Found: C, 49.26; H, 3.95; N, 8.19.

1-(2-Chloro-6-nitrobenzyl)-2,3-dioxopyrrolidine (VI). A mixture of 15.0 g. (0.044 mole) of 1-(2-chloro-6-nitrobenzyl)-4-carbethoxy-2,3-dioxopyrrolidine (V), 15 ml. of 48% hydrobromic acid, and 100 ml. of glacial acetic acid was refluxed for 55 min. The dark solution was poured onto 400 g. of ice. Approximately 6 g. of starting material was recovered by filtration after the ice had melted. The filtrate was extracted with three 150-ml. portions of chloroform, and the combined extracts were dried over anhydrous magnesium sulfate, then filtered, and concentrated by evaporation under reduced pressure. After removal of the chloroform, dry ether (*ca.* 25 ml.) was added to the residual oil and the mixture was kept overnight in a refrigerator. The product, a tan precipitate, was removed by filtration. The yield was 5.28 g. (74.5% yield, 44.7% conversion) of a material melting at 137–140° with previous softening.

Because pyrrolidinediones of this type often undergo condensation reactions during attempted purification, the compound was characterized as the *anil*. To a hot solution of 0.4 g. of the crude product in 5 ml. of absolute ethanol, 0.28 g. of aniline was added, and the solution was boiled for 5 min. The product, which crystallized when the solution was cooled, was washed with a 1:1 absolute ethanol–petroleum ether mixture, then recrystallized twice from ethanol and once from acetone. Yellow, hexagonal prisms were obtained, m.p. 207–209° (dec.).

Anal. Calcd. for $C_{17}H_{14}O_3N_3Cl$: C, 59.39; H, 4.11; N, 12.23. Found: C, 59.14; H, 4.03; N, 12.61.

7-Chlorovasicine (I). Crude 1-(2-chloro-6-nitrobenzyl)-2,3-dioxopyrrolidine (VI) (11.9 g.; 0.044 mole) was dissolved in 200 of warm absolute ethanol, and the solution was added to a mixture prepared by suspending 12.0 g. (0.318 mole) of 98% sodium borohydride in 100 ml. of absolute ethanol. The mixture was allowed to stand at room temperature for 12 hr. and then the solvent was removed by evaporation under reduced pressure. The gelatinous residue was treated with 100 ml. of 25% hydrochloric acid and the mixture was extracted with four 150-ml. portions of chloroform. The combined extract was dried over magnesium sulfate. It was filtered and the solvent was removed by evaporation under reduced pressure.

Because the reduction product, 1-(2-chloro-6-nitrobenzyl)-3-hydroxy-2-oxopyrrolidine (VII), as obtained in this way, was a viscous oil which did not respond to attempts at crystallization, it was not purified but was converted directly into 7-chlorovasicine. To the oil was added 200 ml. of a 50% aqueous acetic acid solution and 18 g. of iron filings. The mixture was stirred and heated on a steam cone for 3 hr. After the mixture had been cooled in an ice bath it was made strongly basic by addition of a 25% aqueous sodium hydroxide solution. The mixture, which was not filtered, was then extracted with three 500-ml. portions of chloroform. (Separation of layers was achieved by centrifugation.) The combined chloroform extract was dried over magnesium sulfate, filtered, and evaporated under reduced pressure. The residue was a pink solid weighing 6.6 g. (68% yield). The infrared spectrum of this material indicated the presence of a small amount of a pyrrolidone impurity. The product was purified by three crystallizations from absolute ethanol using charcoal to decolorize the solutions. The quantity of fully purified product obtained (white needles, m.p. 221–223°) was 1.58 g. (The mother liquors from the crystallizations yielded an additional 0.63 g. of pink needles, m.p. 217–219°, raising the yield of crystalline product to 2.21 g. (22.6%)).

Anal. Calcd. for $C_{11}H_{11}ON_2Cl$: C, 59.33; H, 4.98; N, 12.58. Found: C, 59.39; H, 4.97; N, 12.60.

In the 5.5–7.0μ region the infrared spectrum of the compound ($CHCl_3$ soln.) revealed bands at 6.10μ (20% transmittance), 6.25μ (32% transmittance), 6.39μ (56% transmittance), 6.66μ (61% transmittance), and 6.87μ (38% transmittance). The measurements were made with a Perkin-Elmer model 21 spectrophotometer.

Acknowledgment. The authors are indebted to the Smith Kline and French Laboratories and the Lilly Research Laboratories for the tests of physiological activity described above.

PITTSBURGH 13, PA.