[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN]

The Nitration of 2-Hydroxy-5,6-diphenylpyrazine and 2-Hydroxy-3,6-diphenylpyrazine

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2-Hydroxy-5,6-diphenylpyrazine is readily nitrated in the 3-position. This was proved by degradation and by reduction to the corresponding hydroxyaminodiphenylpyrazine whose structure was demonstrated by two independent syntheses. It is shown that 2-hydroxy-3,6-diphenylpyrazine is nitrated only under more vigorous conditions, yielding a variety of products.

A notable characteristic of the pyrazine nucleus is its resistance to electrophilic substitution.[2] Arylpyrazines are nitrated in the phenyl groups[3] and no nitration of the nucleus has been reported.

We have found that nitration of 2-hydroxy-5,6-diphenylpyrazine in acetic acid yields an easily-purified mononitro compound whose solubility in sodium bicarbonate immediately suggested an *o*-nitrophenol. However, nitration of a phenyl group was not improbable, and it was not known if hydroxypyrazines survive nitrating conditions. A rigorous structural proof of our presumed 2-hydroxy-3-nitro-5,6-diphenylpyrazine was considered necessary.

Oxidation of both 2-hydroxy-5,6-diphenylpyrazine and its nitro derivative with alkaline permanganate yields the same three products in roughly the same amounts—30–35% benzoic acid, 4–5% benzamide, and 20–30% of an unidentified solid. No trace of a nitrobenzoic acid could be found, but since less than half of the phenyl groups were accounted for this did not prove that a phenyl group of the pyrazine had not been nitrated.

Success in cleaving the nucleus of the nitrated pyrazine was achieved by heating it at 82° in 72% sulfuric acid. In this reaction nitrogen oxides and a small amount of carbon dioxide were evolved and there was isolated 24% of benzil, 41% of a high-melting solid whose analysis corresponds to 2,3-dihydroxy-5,6-diphenylpyrazine, and 20% of an unidentified solid. The formation of benzil under these conditions was proof that neither of the phenyl groups in the compound was nitrated.

When the nitro compound was refluxed with hydrazine and Raney nickel in methanol[4] it was reduced to a product which corresponded analytically to a hydroxyaminodiphenylpyrazine (I). Two independent syntheses of this amine conclusively proved its structure. Hoffmann rearrangement[5] of 2-hydroxy-3-carboxamido-5,6-diphenylpyrazine[6] yielded a hydroxyamine II with the same melting point as I. The action of phenyldiazonium hydroxide on 2-hydroxy-5,6-diphenylpyrazine yielded a small amount of the 3-phenylazo coupling product (not isolated in a pure state) which was catalytically reduced to the corresponding hydroxyamine III. These three amines appeared to be identical on the basis of mixed melting points, with I and III being slightly less pure than II. This was confirmed by the analytical data and by the ultraviolet absorption spectra, which differ only in extinction coefficient. It can hardly be doubted that 2-hydroxy-3-nitro-5,6-diphenylpyrazine had been reduced to its 3-amino analog I.

One other nitration of a hydroxypyrazine is mentioned in the literature. Minovici[7] reported only that the action of fuming nitric acid at room temperature on a compound of unknown structure yielded a mononitro derivative (m.p. 232°) which was almost insoluble in the common solvents. It was later suggested[8] that this was a 5-nitropyrazine, and it has recently been proved[9] that the compound which Minovici nitrated was 2-hydroxy-3,6-diphenylpyrazine (IV).

Three grams of the pyrazine IV was at our disposal, and so we have made a brief investigation of its nitration. This hydroxypyrazine was not nitrated at 25° in acetic acid as is the 5,6-diphenyl isomer, but it was rapidly nitrated in fuming nitric acid at 25° and also at 0°. No pure products could be isolated, but two distinct fractions were separated on the basis of solubilities. The major fraction was insoluble in alcohol, acetone and aqueous sodium bicarbonate, and after recrystallization from a large amount of acetic acid it melted at 295–310°. Since nitrogen analysis suggested a *mononitro*hydroxydiphenylpyrazine, we assume that this is the product obtained by Minovici, regardless of the melting point which he reported.[10] A minor fraction from the nitration was fairly soluble in organic solvents and *was soluble* in sodium bicarbonate. After recrystallization from methanol it melted at 196–200° and its analysis indicated a *dinitro* compound. These properties would be expected of a hydroxypyrazine bearing a nitro group in the nucleus. The amounts of these fractions were too small for structure proof, but we suggest that they consist largely of V and VI.

When the hydroxypyrazine IV was dissolved without cooling in fuming nitric acid the reaction was noticeably exothermic, and in addition to V there was isolated another fraction which was soluble in acetone and in sodium bicarbonate, and which melted at 241–247° after two crystallizations from ethanol. On the basis of its nitrogen content and solubilities we suggest the trinitro structure VII.

Treatment of IV in sulfuric acid at 0° with one

(1) Ortho Pharmaceutical Corporation, Raritan, New Jersey.

(2) I. J. Krems and P. E. Spoerri, *Chem. Revs.*, **40**, 328 (1947).

(3) E. Braun and V. Meyer, *Ber.*, **21**, 1269 (1888); E. Ochiai, *et al.*, *J. Pharm. Soc. Japan*, **59**, 462 (1939).

(4) D. Balcom and A. Furst THIS JOURNAL, **75**, 4334 1953.

(5) F. G. McDonald and R. C. Ellingson, *ibid.*, **69**, 1034 (1947).

(6) R. G. Jones, *ibid.*, **71**, 78 (1949).

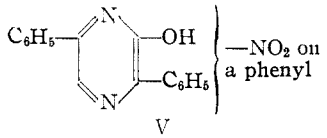
(7) S. Minovici, *Ber.*, **32**, 2206 (1899).

(8) B. Ingham, *J. Chem. Soc.*, 692 (1927).

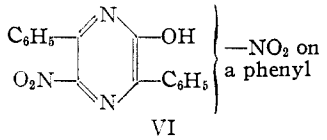
(9) J. J. Gallagher, G. T. Mewbold, F. S. Spring and J. C. Woods, *ibid.*, 910 (1949).

(10) Minovici reported a melting point of 232° but this may be meaningless, for he also reported that the monobromo derivative of 2-hydroxy-3,6-diphenylpyrazine melts at 200°. In ref. 9 it is stated that the bromo compound melts at 246°.

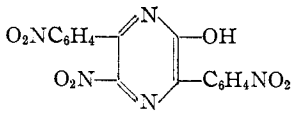
equivalent of nitric acid yielded V and also a very small amount of a bicarbonate-soluble compound as large yellow granules of m.p. 237–240° after crystallization from benzene and from acetone. There was only enough of this for a single nitrogen determination but this figure was close to the theoretical for a mononitro compound and we believe that it was 2-hydroxy-5-nitro-3,6-diphenylpyrazine (VIII).



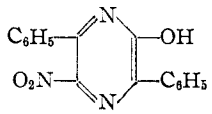
V



VI



VII



VIII

Our investigation has shown that the nitration of 2-hydroxy-3,6-diphenylpyrazine occurs in a random fashion and it seems highly improbable that Minovici could have obtained the 5-nitro derivative simply by recrystallization, as he implied.

Acknowledgments.—We express our sincere thanks to Mr. Joseph Grodsky for all the analyses, to Mr. Alexander Mebane for ultraviolet absorption spectra, and to Dr. R. G. Jones of Eli Lilly and Company for a generous sample of aminomalonamide.

Experimental

I. 2-Hydroxy-3-nitro-5,6-diphenylpyrazine.—A solution of 10 g. (0.04 mole) of 2-hydroxy-5,6-diphenylpyrazine[6,11] in 150 ml. of acetic acid (warm to dissolve) was stirred at 15–20° while 18 ml. of nitric acid (d. 1.5) was added within one minute. After standing in a water-bath at 25° for 1.5 hr. the red solution was poured into 600 ml. of water, and then the yellow solid was filtered off, washed with water, and dried in air. The crude nitro compound was dissolved in 400 ml. of acetone and filtered to remove a small amount of white solid. The filtrate was concentrated to 200 ml. and water was added to the boiling solution until crystallization commenced. Chilling yielded 7.2 g. of yellow prisms, m.p. 210–213°, with decomposition, on a preheated block. A second crop from the mother liquor brought the yield to 8.2 g. (69%).

Anal. Calcd. for $C_{16}H_{11}N_3O_3$: C, 65.52; H, 3.78; N, 14.33. Found: C, 65.88; H, 3.79; N, 14.27.

II. 2-Hydroxy-3-amino-5,6-diphenylpyrazine. Method A.—A mixture of 2 g. (0.01 mole) of 2-hydroxy-3-nitro-5,6-diphenylpyrazine, 1.2 ml. of 85% hydrazine hydrate and 0.1 g. of Raney nickel in 150 ml. of methanol[4] was refluxed with stirring for 1.5 hr. After addition of 350 ml. of water, the dark green solid was filtered off, dried, leached with 400 ml. of boiling butanol, and filtered hot after adding 2 g. of charcoal and 3 g. of Super-Cel. The filtrate was rapidly boiled down to 150 ml. and then cooled slowly to yield 1.2 g. (67%) of tiny, pale yellow prisms, m.p. 307–309° (preheated block), ϵ_{max}^{332} 14,200.

Anal. Calcd. for $C_{16}H_{13}N_3O$: C, 72.97; H, 4.97; N, 15.96. Found: C, 72.76; H, 5.05; N, 15.99.

Method B.—To a solution of 33 g. of potassium hydroxide in 600 ml. of water was added 4 ml. of bromine and then 16 g. (0.055 mole) of 2-hydroxy-3-carboxamido-5,6-diphenylpyrazine.[6] The mixture was stirred at 85° for 2.5 hr. and then it was neutralized at 70° by bubbling in carbon dioxide gas to precipitate the hydroxyaminopyrazine. This was filtered off while hot, dried and recrystallized from one liter of butanol to yield 9.0 g. (62%) of small white prisms, m.p. 309–311°, ϵ_{max}^{331} 14,600.

Anal. Found: C, 72.97; H, 4.90; N, 15.79.

Method C.—A filtered solution of 5 g. (0.02 mole) of 2-hydroxy-5,6-diphenylpyrazine and 8 g. of sodium carbonate (anhyd.) in 1200 ml. of boiling water was vigorously stirred and chilled very rapidly to 20°, using an alcohol–Dry Ice-bath. To this was added all at once 0.02 mole of phenyldiazonium chloride (from 1.8 ml. of aniline plus 6 ml. of 12 *N* hydrochloric acid in 60 ml. of water and 1.4 g. of sodium nitrite in 5 ml. of water). The reaction mixture was stirred at 10° for 20 min. and then it was brought to *p*H 7 with carbon dioxide gas. The red solid was filtered off and dried. Its principal component was the starting material, but by leaching with 200 ml. of hot methanol, concentration of the methanol to 50 ml. under vacuum, and filtration to remove more 2-hydroxy-5,6-diphenylpyrazine, there was obtained a red solution containing a small amount of 2-hydroxy-3-phenylazo-5,6-diphenylpyrazine and the self-condensation products from the diazonium salt. This was diluted to 250 ml. with methanol and was shaken with 1 g. of Raney nickel under 30 lb. of hydrogen for one hour. After filtration, the solution was concentrated to dryness and the tacky residue was leached with 35 ml. of methanol at 25° and filtered to remove 0.3 g. of buff solid. This was dissolved in 150 ml. of warm (45°) 2% potassium hydroxide and the solution was decolorized with charcoal. Neutralization with carbon dioxide precipitated the hydroxyaminopyrazine, which was crystallized from 15 ml. of butanol to yield 0.1 g. of cream-colored prisms, m.p. 304–308°, ϵ_{max}^{332} 13,900.

Anal. Found: C, 72.44; H, 5.26; N, 15.05.

III. Acid Hydrolysis of 2-Hydroxy-3-nitro-5,6-diphenylpyrazine.—To a solution of 3 g. (0.01 mole) of the nitro compound in 100 ml. of sulfuric acid was added, dropwise with stirring, 55 ml. of water, the temperature being kept below 75°, and then the solution was stirred at 82–84° for 1.5 hr. There was a steady evolution of nitrogen oxides (plus some carbon dioxide) as the color changed from orange to pale yellow. The mixture was poured into 700 ml. of cold water and the solids were filtered off and dried. Evaporation of an ether extract of the filtrate gave a small additional amount of solid. The combined solids were first leached with 80 ml. of hot ligroin (b.p. 70–90°) and there was obtained 0.5 g. (24%) of benzil, m.p. 87–89°, on evaporation of this extract. Leaching of the ligroin-insoluble solids with 25 ml. of acetone at 25° yielded a second compound on evaporation of the acetone. This was recrystallized from 6 ml. of ethanol to give 0.6 g. of fine cream flakes, m.p. 280–282° (this compound has not yet been identified). The third solid, insoluble in ligroin and acetone, was recrystallized by dissolving it in 12 ml. of hot acetic acid and diluting with 12 ml. of water. There was obtained 1.1 g. (41%) of small, pale yellow prisms, m.p. 335–340°. This compound corresponds analytically to 2,3-dihydroxy-5,6-diphenylpyrazine.

Anal. Calcd. for $C_{16}H_{12}O_2N_2$: C, 72.67; H, 4.58; N, 10.60. Found: C, 72.44; H, 4.56; N, 10.59.

IV. Nitration of 2-Hydroxy-3,6-diphenylpyrazine. A.—To 10 ml. of nitric acid (d. 1.5) maintained at 20° was added portionwise 0.5 g. of the hydroxypyrazine,[9] and after seven minutes the solution was poured into 200 ml. of water. After filtration and drying the yellow solid was leached with 80 ml. of methanol at 25° and then the extract was concentrated to dryness and the residue was recrystallized from 3 ml. of methanol to give 0.1 g. of bright yellow granules, m.p. 196–200° (with slight cloudiness due to a contaminant). This fraction VI was soluble in aqueous sodium bicarbonate and assayed N 16.16%. Calcd. for $C_{16}H_{10}N_2O(NO_2)_2$: N, 16.56.

The methanol-insoluble material was recrystallized from 75 ml. of acetic acid, giving 0.3 g. of the fraction V. This was insoluble in sodium bicarbonate and contained 13.47% N. Calcd. for $C_{16}H_{11}N_2O(NO_2)_1$: N, 14.34.

B.—To 10 ml. of nitric acid (d 1.5) at 25° was added in one portion 0.7 g. of the hydroxypyrazine. There was a rapid temperature rise and nitrogen oxides were evolved, so the solution was poured into 200 ml. of water after 30

(11) G. Karmas and P. E. Spoerri, THIS JOURNAL, **74**, 1580 (1952).

seconds. After filtration and drying the yellow solid was leached with 60 ml. of hot acetone. This extract was concentrated to dryness and the residue was then leached with 20 ml. of acetone at 25°. The residue from evaporation of this extract was recrystallized twice from 6 ml. of ethanol to give 0.15 g. of yellow prisms, m.p. 241–247° (cloudy). This fraction (VII) was soluble in sodium bicarbonate and contained 17.47% N. Calcd. for $C_{16}H_9N_2O(NO_2)_3$: N, 18.27.

C.—To 0.5 g. of the hydroxypyrazine in 20 ml. of sulfuric acid at 0° was added over ten minutes 0.09 ml. of nitric acid (d. 1.5) in 10 ml. of sulfuric acid. After 15 minutes at 0° the solution was poured into 200 ml. of water and the yellow solid was filtered off. This was boiled a few minutes in 300 ml. of 1% aqueous sodium bicarbonate and after cooling to 25° the mixture was filtered. Acidification of the yellow filtrate precipitated a small amount of solid which was leached with 8 ml. of methanol at 25°. The residue from evaporation of the methanol was recrystallized from 4 ml. of benzene and then from 1.5 ml. of acetone to yield 6 mg. of large yellow granules, m.p. 237–240°. Presumably this is 2-hydroxy-5-nitro-3,6-diphenylpyrazine (VIII).

Anal. Calcd. for $C_{16}H_{11}N_3O_3$: N, 14.33. Found: N, 14.60.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY, AND THE DEPARTMENT OF PHARMACOLOGY, HARVARD MEDICAL SCHOOL]

Schoenocaulon Alkaloids. I. Active Principles of *Schoenocaulon officinale*. Cevacine and Protocevine[1,2]

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On the basis of analogies drawn from the behavior of zygadenine and its esters upon treatment with alkali, the existence of an isomeric, carbonyl-free cevagenine precursor was postulated. In a search for such a precursor, the amorphous fraction of commercial veratrine after removal of cevadine and veratridine was investigated. Fractionation using chromatographic procedures led to isolation of a new alkamine, protocevine, $C_{27}H_{43}O_3N$, isomeric with cevagenine and cevine and a new ester alkaloid, cevacine, $C_{29}H_{45}O_9N$, a monoacetate ester of protocevine. Cevacine yields protocevine upon methanolysis, and protocevine is isomerized to cevagenine by mild alkaline treatment. Acetylation of protocevine with acetic anhydride and pyridine yields protocevine triacetate and similar acetylation of cevacine affords the same triester. Acetylation of protocevine with acetic anhydride and perchloric acid yields anhydroprotocevine tetraacetate, and cevadine has been shown to give an analogous tetraester (anhydrocevadine triacetate) under the same conditions. Protocevine can be obtained from cevadine by methanolysis or by alkaline hydrolysis under very mild conditions. Very mild alkaline hydrolysis of veratridine also yields protocevine. It is proposed that protocevine, and not cevagenine, is the parent alkamine of cevacine, cevadine and veratridine.

Commercial veratrine consists of a mixture of alkaloids obtained from the seeds of *Schoenocaulon officinale*, Gray. Two well characterized ester alkaloids, cevadine and veratridine, have been isolated from the mixture, and these esters yield angelic acid and veratric acid, respectively, on alkaline hydrolysis. Until recently, the alkamine present in cevadine and veratridine was generally believed to be cevine, since this was the only alkamine previously obtainable from the esters by hydrolysis.[5]

Stoll and Seebeck[5] showed, in 1952, that cevadine and veratridine yield cevagenine, a carbonyl-containing isomer of cevine, upon hydrolysis under mild alkaline conditions. They showed further that cevagenine is converted into cevine by treatment with strong alkali (20% alcoholic potassium hydroxide). On the basis of these facts and the evidence that both cevadine and cevagenine show carbonyl absorption at 5.86 μ, those authors concluded that cevagenine, not cevine, is the "genuine" alkamine of cevadine and veratridine.

In a recent paper[6] we have described the relationships between the esters veratroylzygadenine and vanilloylzygadenine and the alkamine zygadenine. Upon mild alkaline treatment, veratroylzygadenine and zygadenine are converted first to an amorphous carbonyl-containing alkamine isomeric with zygadenine. By stronger alkaline treatment, with alcoholic sodium hydroxide, the amorphous alkamine is converted to a second zygadenine isomer, pseudozygadenine, which is carbonyl-free.

The pharmacodynamic action of veratroylzygadenine is similar to that of veratridine.[7] There is also a resemblance between the infrared spectra of the two esters, particularly in the region of 9 to 11.5 μ. Furthermore, the respective products of strong alkaline treatment, pseudozygadenine and cevine, show characteristic and similar absorption properties in the 9 to 11.5 μ region (compare Fig. 1 with Fig. 1 in reference 6). These facts suggested a parallel between the two alkaloidal series, and led us to speculate that a carbonyl-free cevagenine precursor might exist, which would occupy a position in the schoenocaulon series analogous to that of zygadenine in the zygadenus series.

The contrasting behavior of cevadine and cevagenine upon acetylation[8] cast doubt upon the proposal that cevadine is an angelic acid ester of cevagenine. When cevagenine is treated with acetic anhydride and pyridine at steam-bath temperature, dehydration accompanies acetylation, and anhydrocevagenine triacetate is obtained. Treatment

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(2) Presented, in part, at the 123rd Meeting of the American Chemical Society, Los Angeles, Calif., March 17, 1953 (Abstracts, p. 29M).

(3) On leave of absence from the Weizmann Institute of Science, Rehovoth, Israel.

(4) Haffkine Institute, Bombay, India.

(5) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **35**, 1270 (1952). An excellent, concise summary of the literature on the hydrolysis of cevadine and veratridine is given in the introduction to this paper.

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(8) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **35**, 1942 (1952).