Synthesis of 5,6-Dihydro-6-methoxynitidine and a Practical Preparation of Nitidine Chloride

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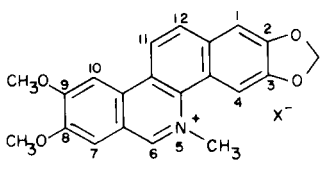
The structures of nitidine (Ia; 8,9-dimethoxy-5-methyl-2,3-methylenedioxybenzo[*c*]phenanthridinium hydroxide) and related alkaloids isolated from various plant extracts (1-11) were initially elucidated by chemical conversion, substantiated by synthesis of dihydronitidine (12,13), and confirmed by nmr studies (14). Other related alkoxybenzo[*c*]phenanthridines such as chelerythrine (15), sanguinarine (16), bocconoline (17), chelidonine (16) and avicine (18), *etc.*, have also been isolated from a variety of plants (19,20).

Recently nitridine chloride (Ib) and 5,6-dihydro-6-methoxynitidine (II) were shown to be highly cytotoxic and displayed antileukemic activity against leukemias P-388 and L-1210 (21) in mice. Additional quantities of Ib and II were required for further biological evaluation. Since these plants are not readily available (22), and since structure I and that of another antileukemic alkaloid, coralyne (III) (23), conform to a proposed *N-O-O* triangular pharmacophore hypothesis for antileukemic activity (24), a search for a practical synthesis of these alkaloids was initiated in this laboratory.

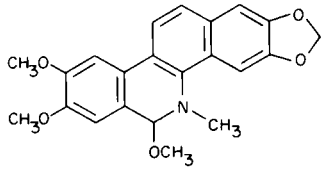
Although a synthetic route leading to benzo[*c*]phenanthridine has been reported (25), the low yield in a Pschorr-type synthesis made this method impractical. Our present synthetic study for the nitidine derivatives follows the method of Arthur and Ng (12) for the synthesis of dihydronitidine with modification to overcome, among others, two major obstacles in the original synthetic route: the formation of the tetralone VII and a Leuckart reaction leading to the formamide derivative VIII. These modifications are summarized as follows: (a) A one-step base hydrolysis of the ketonitrile Va to the ketoacid Vb was achieved in better yield than the original two-step hydrolysis *via* a ketoamide; (b) the yield of the tetralone VII was improved by cyclization of the butyric acid VI at lower temperature with phosphorus pentachloride-stannic chloride rather than refluxing the acid with phosphorus oxychloride; (c) heating the Leuckart reaction mixture initially at higher temperature followed by a longer time at lower temperature gave better results than the conventional Leuckart reaction conditions, and (d) aromatization of the tetrahydrobenzo[*c*]phenanthridine IX to X was accomplished with 30% palladium-on-charcoal in Dow Corning 550 Fluid (DC550). The last modification is particularly useful for large scale manipulation and does not produce a large amount of the undesired 11,12-dihydrobenzo[*c*]phenanthridine XI. The overall yield of nitidine methylsulfate (Ic), based on acetopiperone used, was 15%.

Nitidine chloride (Ib) was obtained by treatment of the methylsulfate salt Ic with aqueous sodium chloride solution. Although this conversion is somewhat similar to that in the coralyne series (23), nitidine methylsulfate (Ic) is much less stable in water than coralyne sulfoacetate. It was necessary to employ low temperatures for the present conversion. The yield of Ib from Ic was 95%.

Treatment of Ib or Ic with concentrated aqueous ammonia, followed by extraction of the resulting mixture with chloroform yielded, after evaporation of solvent, a residual solid. This, when heated with the methanol, furnished the desired new alkaloid II. The synthetic compound thus obtained was identical in every respect



Ia. X = OH
b. X = Cl
c. X = CH_3SO_4



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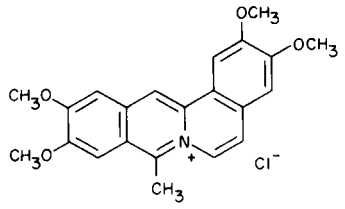

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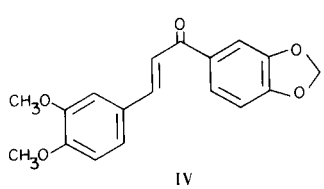

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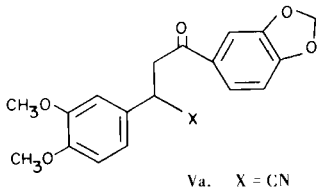
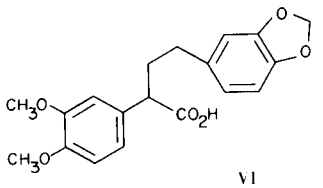


Va. X = CN
b. X = CO_2H



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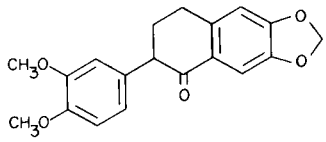

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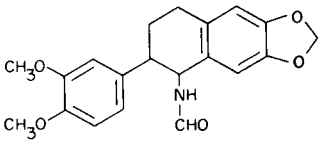
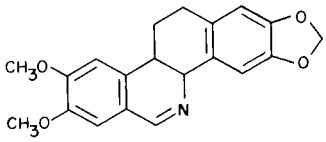
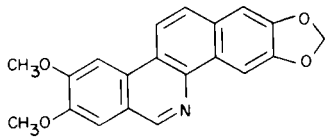


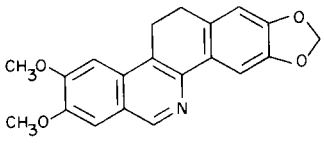
VIII



IX



X



XI

with the plant product provided by the National Cancer Institute.

EXPERIMENTAL

3,4-Dimethoxy-3′,4′-methylenedioxychalcone (IV).

This compound was prepared by the procedure of Arthur and Ng (12) except that an additional amount (30%) of 10% sodium hydroxide was used. The reaction mixture was warmed at 80-90° for 10 minutes followed by overnight standing at room temperature. The yield was raised from 63% to 93%, m.p. 133-135°; λ max (ethanol) 254 (log ε 4.20), 360 nm (4.45).

α-(3,4-Dimethoxyphenyl)-γ-(3,4-methylenedioxyphenyl)-γ-oxobutyric acid (Vb).

A mixture of 76 g. (0.22 mole) of α-(3,4-dimethoxyphenyl)-γ-(3,4-methylenedioxyphenyl)-γ-oxobutyronitrile (Va) (12) and 85 g. (2.13 moles) of sodium hydroxide in 940 ml. of water and 330 ml. of ethanol was refluxed on a steam bath for 10 hours. It was then cooled and acidified with stirring, with 10% hydrochloric acid to *p*H 1. The resulting solid was collected by filtration, washed with water, and dried to give 77 g. (95% yield) of Vb, m.p. 168-171°. Recrystallization from ethanol, m.p. 178-179° (lit., m.p. 172° (12), 188-189° (13)); λ max (ethanol) 230 (log ε 4.41), 274 (3.97) and 307 nm (3.92).

2-(3,4-Dimethoxyphenyl)-1,2,3,4-tetrahydro-6,7-methylenedioxy-1-oxonaphthalene (VII).

A mixture of 20 g. (0.056 mole) of Vb and 2.8 g. of 10% Pd/C in 200 ml. of acetic acid in the presence of 1 ml. of 70% perchloric acid was hydrogenated at 55-60° under 4 kg./cm^2 for 2 hours. The resulting mixture was worked up as usual to yield 20 g. (95% yield) of the acid VI as a light brown gummy residue; λ max (ethanol) 234, 283 nm.

To a stirred suspension of 12.0 g. (0.057 mole) of phosphorus pentachloride in 50 ml. of dry chloroform was added a solution of 19.2 g. (0.056 mole) of VI in 160 ml. of chloroform with ice-bath cooling. The mixture was stirred in the ice bath for 2 hours followed by stirring at room temperature for 16 hours. To this was added dropwise, with ice cooling, a solution of 6.7 ml. (0.056 mole) of anhydrous stannic chloride in 20 ml. of chloroform in 30 minutes. The resulting mixture was stirred at ice bath temperature for 3 hours, then was poured into a mixture of 300 ml. of 10% hydrochloric acid and 200 g. of ice. The gummy complex in the aqueous mixture was gradually dispersed and decomposed by stirring. The mixture was extracted with chloroform (4 x 400 ml.). The resulting chloroform extracts were washed successively with water, 3% sodium hydroxide, and water. The washings were back extracted with chloroform. The combined chloroform extracts were dried (sodium sulfate) and solvent removed *in vacuo*. The residue was triturated with 20 ml. of methanol and the solid was collected by filtration to give 15.5 g. (84% yield) of VII, m.p. 167-170°. Recrystallization from methanol yielded white needles, m.p. 170-172° (lit., m.p. 165° (12), 171-172° (13)); λ max (ethanol), 232 (log ε 4.41), 275 (4.05) and 318 nm (3.97). m/e: 326 (M^+).

2-(3,4-Dimethoxyphenyl)-1-formamido-1,2,3,4-tetrahydro-6,7-methylenedioxynaphthalene (VIII).

To a stirred mixture of 24 g. (0.074 mole) of VII, 60 ml. of redistilled formamide and 3.5 ml. of formic acid was added 3.5 g. of ammonium sulfate. The reaction mixture was heated in an oil bath at 180-182° for 6 hours with stirring. Cautious addition of one 3.5 ml. portion of formic acid to the mixture was made each hour and there was a total of five such additions. The resulting mixture was kept at 140-145° for 12 hours. To the cooled mixture was added 100 ml. of water and 250 ml. of chloroform. After being stirred for 15 minutes, the aqueous layer was separated and extracted with chloroform (3 x 400 ml.); the extract was combined with the organic layer, washed with water, dried (sodium sulfate), and solvent evaporated. The resulting product was purified by column chromatography using neutral

elumina and eluted with chloroform. Evaporation of chloroform eluate yielded a residue, which, when triturated with 20 ml. of methanol, gave 15.9 g. (55% yield) of VIII, m.p. 168-180°. Recrystallization from methanol raised the melting point to 185-187° (lit., m.p. 178° (12), 187° (13)); λ max (ethanol): 230 (log ε 4.20), 285 nm (3.90), λ sh (ethanol) 293 nm (log ε 3.79). m/e: 355 (M^+).

8,9-Dimethoxy-2,3-methylenedioxy-4b,10b,11,12-tetrahydrobenzo[*c*]phenanthridine (IX).

To a stirred suspension of 7.5 g. (0.021 mole) of VIII in 75 ml. of dry toluene at 115° was added 19 ml. of redistilled phosphorus oxychloride. A yellow crystalline solid separated from the clear solution. The mixture was heated at that temperature for a total of 20 minutes and cooled to 40°. The solid was collected by filtration, washed with toluene and ether, and dried to give 7.8 g. of the crude hydrochloride salt, m.p. 211-213° dec. This was suspended in 70 ml. of warm methanol, basified with methanolic ammonia, and cooled. The resulting white crystals were collected by filtration, washed with methanol, and dried to give 4.7 g. (66% yield) of IX, m.p. 188-190°. Recrystallization from methanol and pyridine yielded white crystals, m.p. 198-200° dec. (lit., m.p. 188-189° (12), 193-194° (13)); λ max (ethanol) 233 (log ε 4.52), 285 nm (4.07). m/e: 337 (M^+).

8,9-Dimethoxy-2,3-methylenedioxybenzo[*c*]phenanthridine (2,3-Dimethoxy-12-methyl[1,3-]benzodioxolo[5,6-*c*]phenanthridine, (X).

A mixture of 1 g. of IX and 0.3 g. of 30% palladium-on-charcoal (26) in 15 ml. of Dow Corning 550 Fluid was heated under nitrogen at 255-260° in an oil bath for 2 hours with stirring. The mixture was cooled and diluted with 50 ml. of chloroform. The solid was removed by filtration and extracted continuously with chloroform. The combined filtrate and extracts were evaporated under reduced pressure to yield a pasty substance. This was triturated with 15 ml. of ethanol. The resulting solid product was collected by filtration, washed with ethanol, and dried to give 0.85 g. (85% yield) of X, m.p. 276-278°. An analytical sample was obtained as white crystals by recrystallization from pyridine and ethanol, m.p. 278-280° dec. (lit., m.p. 273 (12), 277-278° (13)); λ max (ethanol) 229 (log ε 4.36), 274 (4.73), 311 (4.15), 348 (3.60), and 367 nm (3.46); λ sh (ethanol) 278 (4.71), 330 nm (3.89); tlc: Rf: 0.48 (chloroform, silica gel) Rf: 0.75 (chloroform, alumina); m/e: 333 (M^+), 335 (M^+ + 2H, trace). The product was found to be identical with that prepared by the previously reported "dry-heating" method (12). Aromatization of IX to X was also accomplished by heating a mixture of IX and sulfur. The yield was comparable.

Attempted aromatization of IX with palladium-on-charcoal in quinoline or with diphenyl disulfide yielded a solid, m.p. 231-233°, which was identified as the 11,12-dihydronitidine derivative XI; λ max (chloroform) 242 (log ε 4.28), 270 (4.52), 277 (4.60) and 326 nm (4.34); nmr (d_6DMSO-TMS): 1.25 τ (1P, *s*, ArH), 2.55 τ (1P, *s*, ArH), 2.88 τ (1P, *s*, ArH), 2.97 τ (1P, *s*, ArH), 3.40 τ (1P, *s*, ArH), 4.22 τ (2P, *s*, $-OCH_2O-$), 6.19 τ (3P, *s*, $-OCH_3$), 6.23 τ (3P, *s*, $-OCH_3$), and 7.02-7.19 τ (4P, *m*, methylene hydrogens).

Anal. Calcd. for $C_{20}H_{17}NO_4$ (335.4): C, 71.63; H, 5.11; N, 4.18. Found: C, 71.30; H, 5.09; N, 4.31; m/e: 335 (M^+, 100%).

Nitidine Methylsulfate (Ic).

To a solution of 2.2 g. (0.0066 mole) of X in 25 ml. of xylene and 50 ml. of nitrobenzene at 160° was added 5 ml. of dimethyl sulfate. This was heated at 180-190° for 5 minutes whereupon a yellow solid separated from the reaction mixture. The mixture was cooled and diluted with 300 ml. of ether. The solid was collected by filtration and washed with ether (3 x 100 ml.) to give 2.3 g. (73% yield) of the monohydrate of Ic, m.p. 307-308° dec. Recrystallization from methanol yielded an analytically pure sample, m.p. 310-312° (lit., m.p. 306-307° dec. (12), 307° dec. (13), unanalyzed). The product was dried at 135° *in vacuo* before analysis; λ max (ethanol)230 (log ε 4.38), 272 (4.68), 300 (4.54), 328 (4.50), and 388 nm (4.00), λ sh (ethanol) 280 nm (4.62). No moving spots were noted under either the following examinations: chloroform, silica gel or chloroform, alumina.

Anal. Calcd. for $C_{22}H_{21}NO_8S\cdot H_2O$ (477.5): C, 55.34; H, 4.86; N, 2.93. Found: C, 55.63; H, 4.58; N, 2.98; m/e: 333 (M^+ - dimethyl sulfate-water).

Nitidine Chloride (Ib).

In 100 ml. of warm (70°) water was rapidly dissolved 250 mg. (0.53 mmole) of Ic. It was filtered immediately [the water-insoluble substance (48 mg.), which separated from the aqueous solution, was identified as the unmethylated compound X by comparison of their ir spectra and tlc] into 200 ml. of stirred 15% sodium chloride solution. The light yellow solid, which separated from the saline solution by filtration, was dried and dissolved in 150 ml. of boiling methanol. On cooling, a light, flocculent white solid separated from the solution. It was collected by filtration and dried to yield 30 mg. of solid, m.p. 303-305°. The solid was identified as the hydrochloride salt of X by comparison of its ir spectra with that of an authentic sample.

Anal. Calcd. for $C_{20}H_{15}NO_4\cdot HCl$ (369.8): C, 64.96; H, 4.36; N, 3.79. Found: C, 65.20; H, 4.57; N, 3.79.

The filtrate was concentrated to 15 ml. to give 60 mg. (48% yield, based on starting material used) of Ib as light yellow crystals, m.p. 275-277° dec. (lit. m.p. 275-276° (11)); λ max (methanol): 234 (log ε 4.39), 270 (4.67), 290 (4.62), 299 (4.61), 327 (4.60), and 380 nm (4.07).

The infrared spectrum of our synthetic compound was found to be identical with that of a natural product provided by Drug Development Branch of the National Cancer Institute.

Anal. Calcd. for $C_{21}H_{18}ClNO_4\cdot 2H_2O$ (419.8): C, 60.07; H, 5.28; N, 3.34. Found: C, 60.15; H, 5.24; N, 3.34; m/e: 333 (M^+ - chloromethane - 2 water, 100%), 52 (48%), 50 (chloromethane, 100%).

In aqueous solution, nitidine methylsulfate gradually decomposed to the water insoluble, uncharged compound X after long standing. This undesirable conversion was accelerated at higher reaction temperature. For a large scale preparation of Ib, the following process can be used. A suspension of finely powdered Ic (5 g.) in 1200 ml. of 8% aqueous sodium chloride solution was rapidly stirred at 0-5° for 30 minutes. The resulting light yellow solid was immediatley collected by filtration on a large fritted glass funnel, washed with water (2 x 10 ml.), and dried to give a 95% yield of Ib, m.p. 276-278°.

5,6-Dihydro-6-methoxynitidine (II).

A mixture of finely ground nitidine methylsulfate hydrate (1 g., 2.1 mmoles) in 140 ml. of 28% aqueous ammonia was stirred in an ice bath for 20 minutes. The mixture was extracted with chloroform (3 x 300 ml.). The extract was washed with water and dried (sodium sulfate). The solvent was removed under reduced pressure and the residue extracted with 100 ml. of hot (50°) methanol (some insoluble solid, identified as the parent ring compound X, was separated by filtration). The volume of the solution was reduced to 20 ml. and 0.45 g. (56% yield) of II was

collected as white crystals, m.p. 186-188° (dried at 78° *in vacuo*). Recrystallization from methanol yielded analytically pure sample, m.p. 189-191° (softened at 186°); λ max (chloroform) 238 (log ε 4.53), 283 (4.60) and 310 nm (4.40); λ sh (chloroform) 325 nm (4.29). No moving spots were noted under the following tlc system: chloroform, silica gel. An Rf value of 0.2 was noted with chloroform, alumina. The product showed identical characteristics (m.p., iv, uv, tlc and mass spectrum) with those of the natural product. This compound can also be prepared from nitidine chloride under similar reaction conditions (27). Attempted preparation of II from Ic with either methanolic ammonia or liquid ammonia followed by treatment with methanol was unsuccessful. Prior to analysis, the sample was dried at 25° and 0.5 mm. for 16 hours.

Anal. Calcd. for $C_{22}H_{21}NO_5$ (379.4): C, 69.64; H, 5.58; N, 3.69. Found: C, 70.00; H, 5.70; N, 3.73; m/e: 379 (12.9%, M^+), 348 (100%, M^+-OCH_3), 333 (17%, M^+-OCH_3-CH_3).

Acknowledgments.

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(19) Extracts from other plants of the same genus *Zanthoxylum* were also found to display interesting biological activity. For example, the Taiwan *Zanthoxylum* is bacteriostatic; components of the so-called "The Hoxsey Cancer Cure" include *Z. americum*, *Z. clavaherculis* L. (pricky ash); *Z. schinifolium* Sieb *et* Zucc. was used in China as folk medicine for the treatment of cancer and other diseases; and the plaster of *Z. hermaphroditum* Willd. were recorded to be beneficial for the treatment of malignant sores and tumors. See Ref. 20 and references cited therein.

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