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MINOR ALKALOIDS OF *ASPIDOSPERMA SUBINCANUM* MART.: ISOLATION, STRUCTURE PROOF AND SYNTHESIS OF 1,2-DIHYDRO-ELLIPTICINE, 1,2-DIHYDRO-ELLIPTICINE METHONITRATE AND ELLIPTICINE METHONITRATE

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Abstract– Three alkaloids named in the title were isolated from the Peruvian plant "Quillo-bordon." The structures assigned were confirmed by partial syntheses.

PLANTS of the genus *Aspidosperma* have been searched intensively for alkaloids of possible therapeutic value. In recent years a number of new bases have been isolated, and the structures of some of them are now known.[1-6] An examination of the Peruvian plant "Quillo-bordon" tentatively identified as *Aspidosperma subincanum* Mart.[7] has shown that two major alkaloids present are N-methyl-tetrahydro-ellipticine (X) and ellipticine[8] (XI).

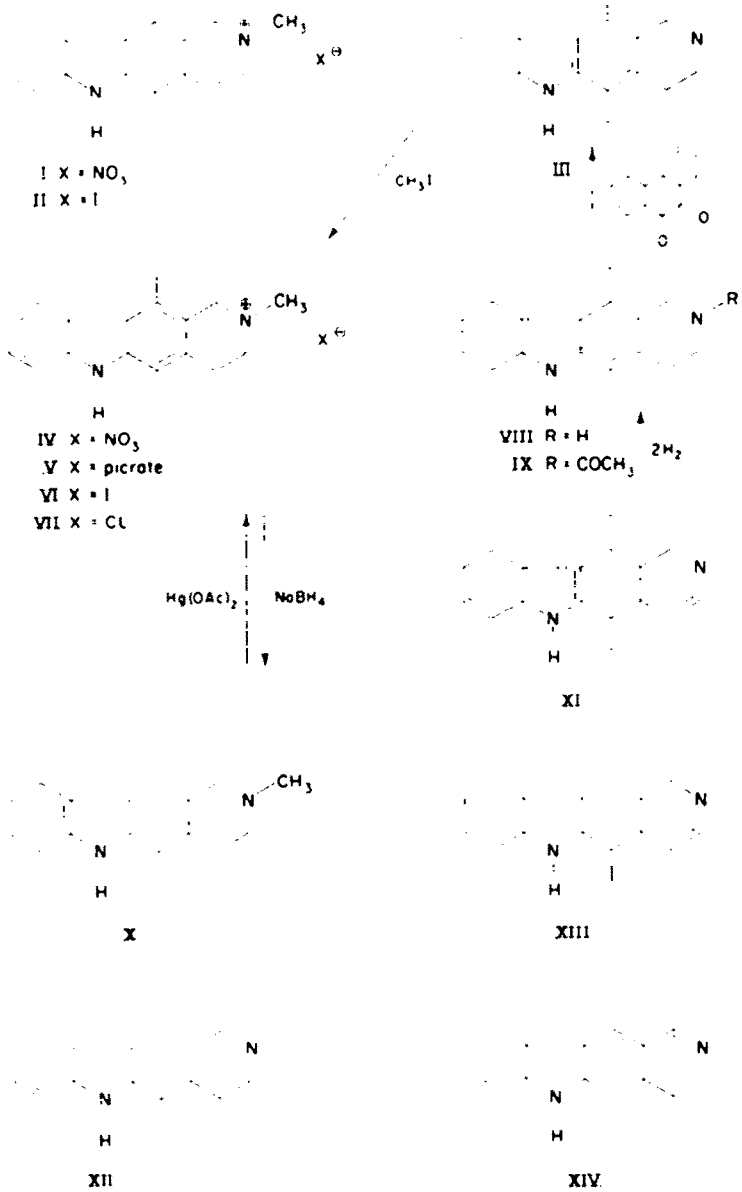
We now wish to record our observations on three minor alkaloids of the same plant and will start with a discussion of the quaternary bases whose presence had been indicated earlier.[8] Crystallization of the crude nitrates from aqueous isopropyl alcohol furnished yellow needles m.p. 300–317° (dec.). Paper chromatographic analysis revealed two major components which were subsequently separated on a preparative scale. The ultraviolet spectrum (see experimental section) of one component m.p. 293–304° (dec.) was essentially identical with that of ellipticine methiodide[3] (II) and indicated the presence of identical cations in the two salts. An authentic sample of ellipticine methonitrate (I) prepared from the methiodide by ion exchange with Dowex-1 (nitrate form) exhibited identical infrared and ultraviolet spectra. Identity was confirmed by R_f values, melting point and mixture melting point comparisons.

Combustion analyses of the second nitrate (IV) and the corresponding picrate (V) both agree with formula $C_{18}H_{19}N_2^{\oplus}$ and the ultraviolet spectrum (see experimental section) of the nitrate is strikingly similar to that of the dihydropyridocarbazole

* National Institutes of Health Postdoctoral Fellow 1960.

[1] H. Lehner and J. Schmutz, *Helv. Chim. Acta* **44**, 444 (1961) and eight earlier papers.
[2] M. A. Ondetti and V. Deulofeu, *Tetrahedron Letters* No. 1, 18 (1960); *Ibid.* No. 7, 1 (1959).
[3] S. Goodwin, A. F. Smith and E. C. Horning, *J. Amer. Chem. Soc.* **81**, 1903 (1959).
[4] B. Gilbert, L. D. Antonaccio, A. A. P. G. Archer and C. Djerassi, *Experientia* **16**, 61 (1960).
[5] R. B. Woodward, G. A. Iacobucci and F. A. Hochstein, *J. Amer. Chem. Soc.* **81**, 4434 (1959).
[6] G. Büchi and E. W. Warnhoff, *J. Amer. Chem. Soc.* **81**, 4433 (1959).
[7] We are indebted to Mr. Allen for providing the plant material which was collected near Iquitos, Peru and to Dr. L. Nickell for the botanical identification.
[8] F. A. Hochstein, A. M. Paradies and R. B. Woodward, unpublished results.

(XIV)[9] in acid solution. Further, strong infrared absorption at 1645 cm $^{-1}$ agrees with the presence of a C $=\overset{\oplus}{N}-$ system. These data indicate that the new alkaloid is either N-methyl-1,2-dihydro-olivacine or N-methyl-1,2-dihydro-ellipticine (IV). Reduction of the nitrate with sodium borohydride in aqueous solution yielded a tertiary base which was identical in every respect with N-methyl-tetrahydro-ellipticine (X). Structure IV was confirmed by a partial synthesis. Oxidation of X with mercuric acetate



[9] G. B. Marini-Bettolo and J. Schmutz, *Helv. Chim. Acta* **42**, 2146 (1959); R. H. F. Manske and M. Kulka, *Canad. J. Res.* **27B**, 291 (1949); *J. Amer. Chem. Soc.* **72**, 4997 (1950).

produced an acetate which was not characterized but converted directly to the corresponding nitrate. This material was identical with the natural salt (IV).

We next investigated the tertiary bases of *Aspidosperma subincanum*. The ultraviolet spectrum of one base (m.p. 293–296°) in acid solution was essentially identical with that of 1,2-dihydro-ellipticine methonitrate (IV). Consequently the two alkaloids contain identical chromophores and we simply had to place a C_2H_5 residue to arrive at the complete structure. Alkylation with methyl iodide in acetone solution furnished a methiodide (VI). This was converted to the corresponding methochloride which was identical in every respect with an authentic sample of 1,2-dihydro-ellipticine methochloride (VII) prepared from the nitrate (IV) by ion exchange. This correlation demonstrates structure III for the new alkaloid. Its structure was confirmed by a partial synthesis. Catalytic reduction of ellipticine (XI) over a platinum catalyst in ethanol solution afforded an air sensitive substance exhibiting ultraviolet light absorption typical of a carbazole. It was characterized by a stable N-acetyl derivative (IX) identified as such by strong infrared absorption at 1630 cm^{-1}. The reduction product, therefore, is 1,2,3,4-tetrahydro-ellipticine (VIII). Dehydrogenation with 9,10-phenanthraquinone in *o*-dichlorobenzene[10] at 120° yielded a yellow substance m.p. 296–300° (dec.). Identity with natural 1,2-dihydro-ellipticine (III) was established by comparison of infrared and ultraviolet spectra, R_f value and mixture melting point.

Both the melting point and infrared spectrum of 1,2-dihydro-ellipticine (III) are different from those reported for *u*-alkaloid D, m.p. 308–312° (dec.) a minor constituent of *Aspidosperma ulei* Mgf.[11] An earlier investigation revealed that this elusive base is a derivative of XIV[9] and when it was found to be different from 1,2-dihydro-olivacine (XII) the investigators assumed its identity with 1,2-dihydro-ellipticine (III).[12] We have sent a small sample of synthetic III to Dr. J. Schmutz, Berne, and he kindly informed us that a careful reexamination of *u*-alkaloid D by thin-layer chromatography on silica gel revealed the presence of two constituents which are identical with 1,2-dihydro-olivacine (XII) and 1,2-dihydro-ellipticine (III) respectively.[13]

In view of the synthesis of ellipticine by Woodward, Iacobucci and Hochstein[5] the partial routes described in this paper may be considered in a formal sense as total syntheses of these new bases.

Finally, we were able to isolate a very small quantity of olivacine (XIII) from the alkaloid fraction of *Aspidosperma subincanum*.

EXPERIMENTAL*

Isolation of 1,2-dihydro-ellipticine methonitrate (IV) *and ellipticine methonitrate* (I). The total dried methanolic extracts of Quillo-bordon bark were dissolved in 20% aqueous methanol, made

* Melting points were observed in evacuated capillaries and are uncorrected. Ultraviolet spectra were obtained with a Beckman DK-2 spectrophotometer. Infrared spectra were recorded with a Baird-Atomic model AB-2 spectrophotometer using a beam condensing unit utilizing silver chloride lenses. Band centers are accurate to 5 cm^{-1} at wave numbers below 1500 cm^{-1} and are followed parenthetically by the observed percent transmission. R_f values are given for two systems: (1) methylethyl ketone saturated with water (MEK) and (2) butanol-acetic acid-water (4:1:5) upper phase (BuOH). Flow rates are also given relative to ellipticine methonitrate (R_e) which was chosen as the standard compound for this series. Standard conditions for paper chromatographic separations were: freshly distilled methylethyl ketone saturated with water, temperature 19°, and Whatman 3 mm paper prewashed with methanol and methylethyl ketone. The elemental analyses were carried out by Scandinavian Microanalytical Laboratory, Copenhagen, Denmark and Dr. S. M. Nagy and associates, M.I.T.

[10] L. M. Jackman in R. A. Raphael, E. C. Taylor, and H. Wynberg, *Advances in Organic Chemistry: Methods and Results* Vol. II, p. 340. Interscience, New York (1960).

[11] J. Schmutz and F. Hunziker, *Helv. Chim. Acta* **41**, 288 (1958).

[12] J. Schmutz and H. Wittwer, *Helv. Chim. Acta* **43**, 793 (1960).

[13] We thank Dr. J. Schmutz for his collaboration. His findings are summarized in ref. 1.

basic and extracted with chloroform to remove tertiary bases. Further extraction of the basic aqueous phase with n-butanol yielded a mixture of crude quaternary bases which was converted to a mixture of yellow crystalline nitrates. Crystallization of the crude nitrates from 2-propanol-water gave bright yellow needles, m.p. 300–317° (dec.). Paper chromatographic analysis of this material employing a system of methylethyl ketone saturated with water indicated the presence of two major components (R_f 0·15 and 0·23). A preparative scale paper chromatographic separation (265 γ/cm) of 80 mg of the crude mixture was carried out. Elution of the component lying between R_f values 0·10 and 0·16 with methanol followed by concentration, filtration and crystallization from methanol gave 6 mg of bright yellow needles. One further crystallization from methanol, an additional paper chromatographic separation (140 γ/cm), isolation and two recrystallizations from methanol afforded (3 mg) ellipticine methonitrate (I), m.p. 293–304° (dec.); R_f^{MEK} 0·15, R_f^{BuOH} 0·71; λ_{max}^{MeOH} 423, 356, 307, 249 and 241 mμ (log ε 3·68, 3·72, 4·86, 4·36 and 4·38 resp.); ν_{max}^{KBr} 1645(53), 1605(17), 1588(41), 1500(60), 1460(39), 1415(11), 1385(2), 1320(7), 1245(14), 1190(35), 1115(52), 825(58), 805(44), 755(20), 715(41).

This material was identical in infrared and ultraviolet spectra, m.p., mixed m.p. and R_f value with an authentic sample of ellipticine methonitrate prepared from ellipticine methiodide[3] by ion exchange with Dowex-1 (nitrate form).

A component lying between R_f values 0·17 and 0·25 was eluted with methanol. The methanol extracts were concentrated under reduced pressure and the residue crystallized from methanol giving 24 mg of pale yellow needles. Two recrystallizations from acetone water followed by two further recrystallizations from methanol gave (15 mg) 1,2-*dihydro-ellipticine methonitrate* (IV), m.p. 301–303° (dec.); R_f^{MEK} 0·23, R_g^{MEK} 1·53; λ_{max}^{MeOH} 382, 313, 302, 281, 271(*sh*), 244(*sh*), and 236 mμ (log ε 4·43, 4·30, 4·18, 4·64, 4·46, 4·30, and 4·45 resp.); ν_{max}^{KBr} 1645(29), 1595(32), 1575(21), 1500(57), 1442(45), 1410(34), 1375(6), 1330(6), 1250(29), 1210(30), 822(60), 778(47), 750(26). (Found: C, 66·61; H, 5·79; N, 13·07. $C_{18}H_{19}N_3O_3$ requires: C, 66·44; H, 5·88; N, 12·91%).

1,2-*Dihydro-ellipticine methopicrate* (V). 1,2-Dihydro-ellipticine methonitrate (100 mg) was dissolved in 5 ml of hot water and mixed with a solution of 100 mg of picric acid in 5 ml of hot water. An immediate amorphous precipitate formed which was filtered and washed with hot water. Three recrystallizations from acetone water afforded (98 mg) 1,2-*dihydro-ellipticine methopicrate* (V) as large bright yellow needles, m.p. 273–275° (dec.). (Found: C, 58·93; H, 4·24; N, 14·22. $C_{24}H_{21}N_6O_7$ requires: C, 58·65; H, 4·31; N, 14·25%).

Isolation of 1,2-*dihydro-ellipticine* (III). A crude fraction (60 mg) obtained during the column chromatography of the residues isolated from the mother liquors remaining from the crystallization of N-methyl-tetrahydro-ellipticine (X)[4] was subjected to further purification. Two successive chromatograms employing "Woelm" almost neutral, activity 1, alumina (elution with chloroform) followed by recrystallization from chloroform–methanol gave (26 mg) 1,2-*dihydro-ellipticine* (III), m.p. 281–283° (dec.). This material was shown to contain a small amount of ellipticine (XI) by paper chromatographic examination.

A small quantity of the partially purified, 1,2-dihydro-ellipticine (3 mg) was subjected to a paper chromatographic separation (100 γ/cm). Methanol was used to elute material between R_f 0·20 and 0·50 (ellipticine R_f 0·65). After removal of the solvent *in vacuo*, the residue was dissolved in 5 ml of 0·1 N HCl, washed with chloroform, made basic (pH 9) with conc NaOH, and extracted exhaustively with chloroform. The chloroform extracts were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The residue was recrystallized twice from methanol to yield (1 mg) 1,2-dihydro-ellipticine (III) as colorless cubes, m.p. 293–296° (dec.).

This material was identical in infrared and ultraviolet spectra, m.p., mixed m.p. and R_f values with an authentic sample of 1,2-dihydro-ellipticine prepared by dehydrogenation of 1,2,3,4-tetrahydro-ellipticine (VIII).

Isolation of olivacine XIII. A second crude fraction (8 mg) also obtained during the column chromatography of the N-methyl-tetrahydro-ellipticine residues[4] was purified by paper chromatographic separation (130 γ/cm). Material lying between R_f 0·50 and 0·65 was extracted and worked up in the usual manner. The residue crystallized when triturated with methanol. Recrystallization first from isopropyl alcohol and finally from methanol afforded (2 mg) of olivacine (XIII) as yellow cubes, m.p. 317–319° (dec.) (lit.[14] 318–324°); R_f^{BuOH} 0·75, R_g^{BuOH} 1·03.

This material (although paper chromatographic examination indicated that traces of ellipticine

[14] J. Schmutz and F. Hunziker, *Pharm. Acta Helv.* 33, 341 (1958).

still persisted in the sample) was identical in infrared spectrum, m.p., mixed m.p. and R_f value with an authentic sample of olivacine kindly given to us by Dr. J. Schmutz.

Sodium borohydride reduction of 1,2-*dihydro-ellipticine methonitrate* (IV). An aqueous solution (2 ml) of 60 mg of sodium borohydride was rapidly added to 30 mg of 1,2-dihydro-ellipticine methonitrate dissolved in 7 ml of water. A yellow amorphous solid immediately precipitated. The reaction mixture was shaken for 30 min and then acidified with dil HCl. The acidic aqueous phase was washed with chloroform, made basic (pH 9) with conc NaOH and extracted with ether. The ether extracts were dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure. Four recrystallizations of the residue afforded (9 mg) N-methyl-tetrahydro-ellipticine[4] as colorless rosettes, m.p. 224·5–225° (lit.[11] 215–218°); R_f^{BuOH} 0·72, R_q^{BuOH} 1·00, R_f^{MEK} 0·23, R_q^{MEK} 1·21; λ_{max}^{MeOH} 340, 327, 317(*sh*), 294, 284(*sh*), 263, 248(*sh*), 242, and 229(*sh*) mμ (log ε 3·56, 3·58, 3·42, 4·26, 3·96, 4·31, 4·57, 4·69 and 4·48 resp.); ν_{max}^{KBr} 1610(16), 1580(48), 1500(35), 1455(14), 1403(34), 1370(15), 1335(21), 1312(14), 1287d(30), 1265(12), 1230(41), 1190(55), 1165(51), 1120t(30), 1075(40), 1012d(54), 983(58), 970(39), 892(43), 820(41), 770(52), 732(10), 705(50).

This material was identical in infrared and ultraviolet spectra, m.p., mixed m.p., and R_f values with an authentic sample of N-methyl-tetrahydro-ellipticine.

Mercuric acetate oxidation of N-*methyl-tetrahydro-ellipticine* (X). N-methyl-tetrahydro-ellipticine (40 mg) was refluxed with 160 mg of mercuric acetate in 3 ml of 5% aqueous acetic acid for 3·5 hr. The initial colorless solution rapidly turned bright yellow in color. After filtration of the mercurous acetate and saturation with hydrogen sulfide the aqueous solution was made strongly acidic with conc HNO_3. The black colloidal precipitate was collected by centrifugation and extracted repeatedly with hot ethanol. On concentration of the ethanol extracts the product crystallized as fine yellow needles. Several recrystallizations from methanol gave (6 mg) 1,2-*dihydro-ellipticine methonitrate* (IV), m.p. 302 307° (dec.).

This material was identical in infrared and ultraviolet spectra, m.p., mixed m.p. and R_f values with the natural salt.

Catalytic reduction of ellipticine (XI). The catalytic reduction of ellipticine appeared to proceed slowly or not at all under a variety of conditions. Reduction over PtO_2 in ethanol, however, could be effected by employing large amounts of catalyst and, although little hydrogen uptake could be detected, the course of the reaction was monitored by observing changes in the ultraviolet spectrum of aliquots withdrawn at regular intervals.

Ellipticine (40 mg) was dissolved in 25 ml of absolute ethanol and hydrogenated over (130 mg) PtO_2 for 12 hr. The colorless reaction mixture was divided into two equal portions. The catalyst was rapidly removed from portion No. 1 by filtration in an atmosphere of nitrogen. Evaporation of the solvent *in vacuo* gave 19 mg of colorless fan shaped crystals which rapidly turned yellow in color on contact with air. Two recrystallizations from methanol afforded (13 mg) 1,2,3,4-*tetrahydro-ellipticine* (VIII), m.p. 160 165° (dec.); R_f^{BuOH} 0.79, R_q^{BuOH} 1·13, R_f^{MEK} 0·30, R_q^{MEK} 1·34; λ_{max}^{MEOH} 341, 327, 316(*sh*), 294, 284(*sh*), 263, 249(*sh*), 242 and 228(*sh*) mμ (log ε values were not obtained, however, the relative extinction coefficients for the absorption maxima were virtually identical to those observed for N-methyl-tetrahydro-ellipticine). As the crystalline material still exhibited great sensitivity toward air, further characterization of the compound was carried out with the acetyl derivative. Portion No. 2 rapidly turned yellow on standing in contact with air and no identifiable products could be isolated after removal of the catalyst.

Acetyl derivative (IX). Acetylation of 1,2,3,4-tetrahydro-ellipticine with acetic anhydride-pyridine followed by several recrystallizations from methanol gave white needles, m.p. 272·5–273°; R_f^{BuOH} 0·86, R_q^{BuOH} 1·23, R_f^{MEK} 0·94, R_q^{MEK} 4·27; λ_{max}^{MeOH} 340, 327, 318(*sh*), 294, 285(*sh*), 263, 248(*sh*), 242 and 229(*sh*) mμ (log ε 3·54, 3·59, 3·45, 4·20, 3·71, 4·30, 4·57, 4·70 and 4·51 resp.); ν_{max}^{KBr} 1630(7), 1612(3), 1492(47), 1455(15), 1420(23), 1361(53), 1338d(62), 1300d(49), 1268(22), 1239(30), 1117(56), 1050(63), 1030d(66), 990d(65), 875(61), 775(58), 740(18). (Found: C, 77·74; H, 6·86; N, 9·61. $C_{19}H_{20}N_2O$ requires: C, 78·04; H, 6·89; N, 9·58%).

Dehydrogenation of 1,2,3,4-*tetrahydro-ellipticine* (VIII). Ellipticine (100 mg) in absolute ethanol (40 ml) was hydrogenated over PtO_2 (154 mg) for 16 hr. After filtration of the catalyst in an atmosphere of nitrogen and removal of the solvent *in vacuo*, the white crystalline product (~70 mg) was immediately dissolved in 70 ml of *o*-dichlorobenzene and rapidly added to a solution of 50 mg of 9,10-phenanthra-quinone in 30 ml of the same solvent. The reaction vessel was quickly flushed with nitrogen, sealed under vacuum and kept at 120–130° for 24 hr. After cooling, the *o*-dichlorobenzene

solution was exhaustively extracted with dil HCl. The acidic aqueous extracts were washed with chloroform, adjusted to (pH 9) with conc NaOH and extracted repeatedly with chloroform. The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure. The residue was crystallized once from methanol and then subjected to paper chromatographic separation (300 γ/cm). Extraction of the material lying between R_f 0·25 and 0·65 with methanol and removal of the solvent *in vacuo* gave a yellow amorphous residue which was taken up in 10 ml of 0·1 N HCl and washed with chloroform. The acidic aqueous phase was made basic (pH 10) and extracted with chloroform. The chloroform extracts were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The residue (26 mg) crystallized on trituration with methanol. Elution with chloroform from "Woelm" almost neutral, activity 1, alumina and three recrystallizations from methanol gave (12 mg) of 1,2-*dihydro-ellipticine* (III), m.p. 296 300° (dec.); R_f^{BuOH} 0·73, R_q^{BuOH} 1·05, R_f^{MEK} 0·34, R_q^{MEK} 1·50; $\lambda_{max}^{0\cdot1\,N\,HCl}$ 378, 312, 301, 279, 270(sh), and 235 mμ (log ε 4·36, 4·28, 4·15, 4·62, 4·45 and 4·42 resp.); γ_{max}^{KBr} 1615(14), 1595(14), 1570(10), 1495(17), 1445(29), 1410(38), 1355(35), 1325(7), 1265(20), 1225(31), 1167(45), 1123(41), 1028(42), 930d(64), 909(43), 843(50), 775(40), 750(10), 660(44). (Found: C, 82·12; H, 6·42; N, 11·36. $C_{17}H_{14}N_2$ requires: C, 82·23; H, 6·49; N, 11·28%).

This material was identical in infrared and ultraviolet spectra, m.p., mixed m.p. and R_f values with the natural base.

Conversion of 1,2-*dihydro-ellipticine* (III) *to* 1,2-*dihydro-ellipticine methochloride* (IV). Natural 1,2-dihydro-ellipticine (20 mg) in acetone (30 ml) was rapidly mixed with methyl iodide (5 ml) in acetone (5 ml) and left at room temperature for 1 hr. The bright yellow crystalline precipitate (29 mg) was filtered, washed with acetone and dissolved in methanol-water 1 : 1 (40 ml). The aqueous-methanolic solution of 1,2-dihydro-ellipticine methiodide was passed over Dowex-1 (2 g) in the chloride form. Removal of the solvent *in vacuo* gave 15 mg of a yellow crystalline residue a portion of which was chromatographed on paper (165 γ/cm). Extraction with methanol of material lying between R_f 0·18 and 0·30, evaporation of the solvent under reduced pressure, and two recrystallizations from methanol-ethyl acetate afforded (7 mg) of 1,2-*dihydro-ellipticine methochloride* (VII), m.p. 296 306° (dec.); R_f^{MEK} 0·185, R_q^{MEK} 1·16; λ_{max}^{MeOH} 383, 314, 302, 282, 271(*sh*), and 236 mμ (log ε 4·40, 4·20, 4·02, 4·62, 4·40 and 4·34 resp.); γ_{max}^{KBr} 1650(29), 1595(30), 1575(21), 1500(59), 1447(48), 1412(49), 1375(49), 1331(21), 1305(50), 1250(45), 1215(45), 1188(52), 1170(57), 1110(64), 778(55), 749(42).

This material was identical in infrared and ultraviolet spectra, m.p., mixed m.p. and R_f value with an authentic sample of 1,2-dihydro-ellipticine methochloride (VII) prepared from 1,2-dihydro-ellipticine methonitrate (IV) by ion exchange with Dowex-1 (chloride form).

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