Isolation of Cephalotaxine from *C. Fortunei*.—Following the procedure previously described for *C. drupacea*, an alcoholic extract of 0.342 kg. of the powdered leaves and stems of *C. fortunei* yielded 1.320 g., 0.39% of crude alkaloidal material. Chromatography of 1.25 g. of this material on alumina yielded 659 mg. of pure crystalline alkaloid (R_f 0.35, m.p. 130–132°). The infrared spectrum of this alkaloid was superimposable upon that of cephalotaxine. A mixture melting point of the two alkaloids was not depressed.

Acetylcephalotaxine.—A solution of 1.0432 g. of cephalotaxine in 8 ml. of acetic anhydride was heated under reflux for 1 hr. The resulting brown solution was evaporated to dryness, leaving the resulting brown oil which was dissolved in chloroform and chromatographed on 50 g. of grade II (Brockman scale) neutral alumina (Arthur H. Thomas Co.). Elution with 100 ml. of ether gave 0.9781 g. of crystalline material (R_f with respect to cephalotaxine, 2.1). The material was recrystallized from ether, m.p. 140–142°. This melting point was not altered by two subsequent recrystallizations. The infrared spectrum of this compond had new absorption peaks at 1735 cm.$^{-1}$ and 1240 cm.$^{-1}$, typical for an acetate, but lacked peaks in the region 3700–3500 cm.$^{-1}$ ascribable to hydroxyl groupings.[10] A portion of the alkaloidal material was prepared for elemental analysis by recrystallization from ether.

Anal. Calcd. for $C_{20}H_{23}NO_5$: C, 67.2; H, 6.45; N, 3.92; O, 22.4; mol. wt., 357. Found: C, 67.3; H, 6.65; N, 3.81; O (by difference), 21.7; mol. wt. in benzene, 356.

Acetylcephalotaxine, m.p. 140–142°; $[\alpha]^{25}D$ −97 (*c* 2.2, chloroform); pK_a 7.97 (95% ethanol). Acetylcephalotaxine decolorized an aqueous solution of potassium permanganate, as well as a solution of bromine in carbon tetrachloride.

Reduction of Acetylcephalotaxine with Lithium Aluminum Hydride.—A solution of 28.7 mg. (0.0804 mmole) of acetylcephalotaxine in 20 ml. of ether was refluxed in the presence of 82.2 mg. of lithium aluminum hydride, for 4 hr. The excess hydride was then decomposed by the slow addition of 1 ml. of water. The resulting emulsion was broken by the addition of 30 ml. of additional ether and the ether layer was collected. The dried ether extract (over anhydrous sodium carbonate) was then evaporated to dryness, leaving 21.8 mg. of a white crystalline solid, whose melting point (131–132°) was not depressed when mixed with cephalotaxine. The infrared spectrum of this compound was superimposable upon that of cephalotaxine.

Acid Hydrolysis of Cephalotaxine.—A solution of 70.3 mg. of cephalotaxine in 1.5 ml. of 1 *N* sulfuric acid was allowed to stand at room temperature for 18 hr. The yellow solution was then made basic by addition of 3.0 ml. of an aqueous sodium bicarbonate solution, and the basic solution was extracted with three 5-ml. portions of chloroform. The dried (over anhydrous sodium carbonate), combined extracts were evaporated to dryness under reduced pressure, leaving 61.0 mg. of white amorphous material. This compound gave a positive ferric chloride test. The infrared spectrum indicates the presence of a carbonyl function (1705 cm.$^{-1}$). A paper chromatogram showed the presence of a trace cephalotaxine (R_f 0.54) and of another alkaloidal constituent (R_f 0.37).

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The Alkaloids of *Hunteria eburnea* Pichon. III.[1] The Tertiary Bases

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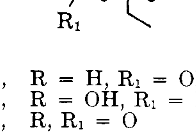
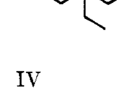
The isolation of nine tertiary bases from *Hunteria eburnea* Pichon is described. Eburnamine (I), isoeburnamine (II), eburnamonine (III), and eburnamenine (IV) are the first members of a new class of pentacyclic indole alkaloids. A new variant of the yohimbinoid ring skeleton is found in burnamicine (VI). Of the remaining alkaloids the indolines pleiocarpine (V) and pleiocarpamine are known compounds, while burnamine, an indoline with an echitamine type ultraviolet absorption spectrum, and neburnamine, an indole, are new compounds.

In the course of investigating extracts of *Hunteria eburnea* Pichon (Apocynaceae) for hypotensive principles,[2] a total of nine alkaloids were isolated from the tertiary base containing fraction.[3] The elucidation and synthesis of the structures of four of these, eburnamine (I),[4] isoeburnamine (II), eburnamonine (III), and eburnamenine (IV), a new class of pentacyclic indole alkaloids, were reported previously.[5] Two others, pleiocarpine (V, R_1 = $COOCH_3$, R_2 = H_2)[6,7] and pleiocarpamine,[6,8] also isolated from both *Pleiocarpa tubicina* and *Hunteria eburnea*[6] were readily recognized. The remaining three, burnamine, burnamicine, and neburnamine, were not available in sufficient amounts for complete structural determination by chemical methods, although preliminary investigation of the former alkaloid proved it to have very interesting properties (*vide infra*). A structure (VI) was proposed for burnamicine, mainly on the basis of its mass spectrogram, the details of which were published separately.[9]



I, R = H, R_1 = OH
II, R = OH, R_1 = H
III, R, R_1 = O



IV

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[1] Previous paper, M. F. Bartlett, B. Korzun, R. Sklar, A. F. Smith, and W. I. Taylor, *J. Org. Chem.*, **28**, 1445 (1963).

[2] (a) Raymond-Hamet, *Compt. rend.*, **240**, 1470 (1955); (b) A. Engelhardt and H. Gelbrecht, *Naturwissenschaften*, **45**, 547 (1958); (c) A. Engelhardt and H. Gelbrecht, *Arzneimittel-Forsch.*, **11**, 414 (1961).

[3] A tenth tertiary base was isolated among the quaternary bases, ref. 1.

[4] Eburnamine and neburnamine were first isolated in trace amounts from a chromatogram of the total bases by D. F. Dickel.

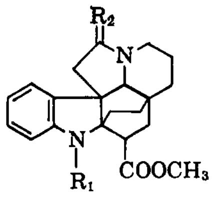
[5] M. F. Bartlett, W. I. Taylor, and Raymond-Hamet, *Compt. rend.*, **249**, 1259 (1959); M. F. Bartlett and W. I. Taylor, *Tetrahedron Letters*, No. **20** 20, (1959); M. F. Bartlett and W. I. Taylor, *J. Am. Chem. Soc.*, **82**, 5941 (1960).

[6] W. G. Kump and H. Schmid, *Helv. Chim. Acta*, **44**, 1503 (1961). According to a paper chromatographic assay, our pleiocarpine contains a little pleiocarpinine (V, R_1 = CH_3, R_2 = H_2; W. G. Kump and H. Schmid, personal communication). These authors also isolated eburnamenine (IV) from *Pleiocarpa mutica*. Eburnamenine was found recently in two other plants, *Rhazya stricta* and *Aspidosperma quebracho*, eburnamonine also being recognized in the former [H. K. Schnoes, A. L. Burlingame, and K. Biemann, *Tetrahedron Letters*, 993 (1962)].

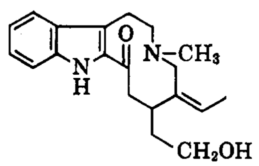
[7] W. G. Kump, D. J. Le Count, A. R. Battersby, and H. Schmid, *Helv. Chim. Acta*, **45**, 854 (1962).

[8] This compound has also been recognized as a product of the acid-catalyzed fission of villalstonine (B. S. Joshi and W. I. Taylor, unpublished observations).

[9] M. F. Bartlett and W. I. Taylor, *J. Am. Chem. Soc.*, **85**, 1203 (1963).



V



VI

For this work, both stem and root bark were percolated with methylene chloride, the crude tertiary bases being separated by extraction through strong acid and further divided into methylene chloride and dilute acetic acid-extractable bases by partitioning between these solvents. From the dilute acetic acid-soluble portion, eburnamine (I), isoeburnamine (II), burnamine, burnamicine (VI), and neburnamine were isolated. Upon chromatography of the methylene chloride extractables, the remaining four alkaloids, eburnamonine (III), eburnamenine (IV), pleiocarpine (V), and pleiocarpamine, were obtained. Hunterine,[10] pleiocarpinine (V, $R_1 = CH_3$, $R_2 = H_2$),[6,7] pleiocarpinilam (V, $R_1 = CH_3$, $R_2 = 0$),[11] kopsinilam (V, $R_1 = H$, $R_2 = 0$),[11] obtained by others from *Hunteria eburnea*, and corymine, from *Hunteria corymbosa*,[12] were not isolated by us.[13]

Burnamine, a dihydroindole with an echitamine-type[14] ultraviolet absorption spectrum (λ_{max} 234 and 288 mμ) and an empirical formula $C_{21}H_{26}N_2O_4$ (equivalent weight, 372 ± 12) contained one methoxyl, no N-methyl, one ethylidine side chain (p.m.r. spectrum), OH (3590 cm.$^{-1}$), and probably NH (3040 cm.$^{-1}$). The carbonyl absorption at 1740 cm.$^{-1}$ suggested the presence of a carbomethoxy group. Upon oxidation with lead tetraacetate, burnamine was transformed into a product $C_{21}H_{24}N_2O_4$, λ_{max} 215 mμ (ϵ 2500), with the carbonyl absorption shifted to 1724 cm.$^{-1}$. Upon catalytic reduction this product reverted to burnamine with the uptake of one mole equivalent of hydrogen. The conversion indoline → indole appeared to be facile, as one or more quaternary indoles were formed by treatment of burnamine with methyl iodide. Unfortunately, paucity of material prevented further investigation of these intriguing results.

The remaining unknown alkaloid, neburnamine, an indole possibly associated with a second chromophore, was obtained only after extensive fractionation of the more polar acetic acid-soluble bases, but consistent elementary analyses were not obtained for this highly oxygenated base. Because the molecule contains two basic nitrogens, pK_a' 7.7 and 9.9, it was assumed to be a dimeric indole. Again lack of material curtailed further work.

Experimental

The melting points were taken *in vacuo* and are uncorrected. Samples were routinely dried at 70–80° for 12–24 hr. for analysis. The ultraviolet absorption spectra were run in alcohol and are expressed in mμ (ϵ), the infrared absorption spectra in cm.$^{-1}$ (Nujol). The potentiometric micro titrations were done in 1:1 methanol–water, the optical rotations in chloroform, and the p.m.r. spectra were run in deuteriochloroform in a Varian HR 60 instrument.

Extraction of *Hunteria eburnea* Bark.—A bed of ground *Hunteria eburnea* stem bark (38 kg.) and root bark (22 kg.) was extracted with recycling methylene chloride at 34.°[15] Upon reduction of the volume to two fractions of *ca.* 3 l. each, the bases were extracted into 25% sulfuric acid, the acidic extract made basic (pH 11) with sodium hydroxide and extracted with methylene chloride, washing with water. After drying (sodium sulfate), evaporation of solvent gave 535 g. of crude tertiary bases. The crude extract was separated into strong and weak bases by partitioning countercurrent distribution style through five portions (2 l. each) of 5% acetic acid with six portions (2 l. each) of methylene chloride. Combined organic phases, after drying (sodium sulfate), gave 119 g. of methylene chloride-soluble bases. The combined aqueous phases, upon making basic with sodium hydroxide and extraction with methylene chloride, yielded 300 g. of acetic acid-soluble bases.

The Acetic Acid-Soluble Bases.—The acetic acid-soluble bases (302 g.) were chromatographed on 1.8 kg. of basic activity III alumina. The 500-ml. fractions of eluate were grouped as follows [(solvent ratios, vol. of eluate), wt. eluted]: benzene–methylene chloride (2:1, 2.5 l.), 10.9 g.; benzene–methylene chloride (2:1, 0.5 l.), 19.0 g.; benzene (2.0 l.), 14.3 g.; benzene (0.5 l.), 4.8 g.; methylene chloride (2.0 l.), 91.2 g.; methylene chloride (8.0 l.), 13.6 g.; and methanol–methylene chloride (1:1, 5.0 l.), 125 g.

Eburnamine (I).—The second benzene eluate (4.8 g.) crystallized from ethanol yielding eburnamine (1.34 g.), m.p. 180–181°, increasing to 186–187° from ethanol; $[\alpha]_D$ −93°; pK_a' 7.7; λ_{max} 229 (33,800), 282 (8100); λ_{sh} 276 (8400); λ_{min} 249 (2500). A sample was sublimed at 140° (0.01 mm.) for analysis.

Anal. Calcd. for $C_{19}H_{24}N_2O$: C, 76.99; H, 8.16; N, 9.45. Found: C, 76.98; H, 8.15; N, 9.19.

Isoeburnamine (II).—The second benzene–methylene chloride eluate (19.0 g.) in ethanol yielded isoeburnamine (2.0 g.), m.p. 202–204°, increasing to 217–220° on recrystallization from methanol; $[\alpha]_D$ +111°; pK_a' 7.8; λ_{max} 229 (34,600), 282 (7900); λ_{sh} 277 (7800); 291 (6800); λ_{min} 248 (2100).

Anal. Calcd. for $C_{19}H_{24}N_2O$: C, 76.99; H, 8.16; N, 9.45. Found: C, 76.47; H, 8.01; N, 9.52.

Rechromatography of the Methylene Chloride Eluate.—A portion (30 g.) of the first methylene chloride eluate was rechromatographed on basic alumina activity IV (60 × 260 mm.), the following eluates being obtained: benzene (1.6 l.), 5.4 g.; benzene (6.0 l.), 7.8 g.; 1:1 benzene–methylene chloride (5 l.), 1.0 g.; methylene chloride (3.0 l.), 1.9 g.; methylene chloride (1.0 l.), 0.4 g.; methylene chloride (3.0 l.), 0.6 g.; 1% methanol in methylene chloride (4.0 l.), 3.2 g.

From the first benzene fraction, isoeburnamine (2.4 g.), m.p. 186–188°, was obtained while eburnamine (4.1 g.), m.p. 180–181°, crystallized from the second benzene eluate.

Burnamine.—From the first methylene chloride fraction of the preceding chromatogram, burnamine (0.4 g.), m.p. 197–198°, crystallized from benzene–ether; $[\alpha]_D$ −131°; pK_a' 6.3; equiv. wt., 372 ± 12; λ_{max} 234 (6800), 288 (3100); λ_{min} 225 (6700), 255 (1000). The spectrum does not change in dilute acid or base; $\nu_{OH|NH}$ 3590, 3120, 3050; ν_{CO} 1740.

Anal. Calcd. for $C_{21}H_{26}N_2O_4$: C, 68.09; H, 7.07; N, 7.56; OCH_3, 8.39. Found: C, 68.23, 68.62, 68.39; H, 6.75, 6.79, 6.67; N, 7.13, 7.31, 7.23; OCH_3, 8.66; NCH_3, 0.0; C–CH_3, 3.6.

This material decomposed upon attempted sublimation *in vacuo* at 150°.

Lead Tetraacetate Oxidation of Burnamine.—Burnamine (130 mg.) suspended in benzene (70 ml.) was treated with lead tetraacetate (270 mg.). After standing for 10 min., the solution was diluted with methylene chloride and extracted with dilute ammonium hydroxide. The residue (160 mg.) obtained from the methylene chloride was then chromatographed on neutral alumina, activity III. The methylene chloride eluate yielded a residue (80 mg.) crystallizing from ether–hexane (60 mg.), m.p. 190–198°, increasing to 198–200° on recrystallization; λ_{max} 315 (2500); λ_{sh} 240 (2750); λ_{min} 269 (1130); ν_{CO} 1725 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{24}N_2O_4$: C, 68.46; H, 6.57; N, 7.60. Found: C, 68.30; H, 6.21; N, 7.59.

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[10] N. Neuss and N. J. Cone, *Experientia*, **16**, 302 (1960).

[11] C. Kump and H. Schmid, *Helv. Chim. Acta*, **45**, 1090 (1962).

[12] A. K. Kiang and G. F. Smith, *Proc. Chem. Soc.*, **298** (1962).

[13] Akuammine, claimed to be in *Hunteria eburnea* by Engelhardt and Gelbrecht (ref. 2c) because of similarities of biological activity between it and an alkaloidal fraction, was not detected in any of our extracts.

[14] T. R. Govindachari and S. Rajappa, *Proc. Chem. Soc.*, 132 (1959).

[15] This part of the extraction was done in our Pilot Plant section by J. W. Drew and L. W. Blodgett.

Hydrogenation of the Oxidation Product.—This oxidation product (11 mg.) was reduced in ethanol (5 ml.) with prereduced platinum oxide (12 mg.). The uptake stopped at one mole equivalent. Upon filtering and evaporating the solvent, the material crystallized from benzene–ether, m.p. 200–202°, identical to **burnamine**.

Burnamicine (VI).—The third methylene chloride fraction (0.6 g.) crystallized from ethanol yielding burnamicine (140 mg.), m.p. 198–200° dec. A sample was sublimed *in vacuo* at 100–145° for analysis; $[\alpha]_D$ $-281°$; pK_a' 8.9; $\lambda_{\max}^{\text{neutral or base}}$ 311(14,600); λ_{sh} 236 (13,500); $\lambda_{\min}$ 265 (5200); $\lambda_{\max}^{\text{acid}}$ 269 (7280), 280 (7000), 290 (5000); $\lambda_{\min}$ 238 (1800), 277 (6700), 289 (4900); $\nu_{OH|NH}$ 3425, 3195.

Anal. Calcd. for $C_{19}H_{24}N_2O_2$: C, 73.04; H, 7.74; N, 8.97; NCH_3, 4.8; act. H, 0.32. Found: C, 73.23, 73.07; H, 8.04, 8.09; N, 8.74; NCH_3, 4.81; OCH_3, 0.00; act. H, 0.63.

Neburnamine.—A portion (68 g.) of the 1:1 methanol–methylene chloride fraction (125 g.) of the first chromatogram was subjected to a twelve-stage countercurrent distribution (1-l. phases) in the system, 0.4 *M* disodium phosphate (2.75 l.), 0.2 *M* citric acid (0.25 l.), methanol (1.34 l.), and methylene chloride (6 l.). The solutions in the first six funnels were combined, made basic with sodium hydroxide, and extracted with methylene chloride yielding 5.1 g. of amorphous material.

Tars (14 g.) that had precipitated in funnels 1 and 2 were removed by extraction with methylene chloride–dilute sodium hydroxide. These were distributed in the system, 0.5 *N* acetic acid (2.4 l.), 0.5 *N* sodium acetate (600 ml.), methanol (1.5 l.), and methylene chloride (5 l.) for six transfers. The material isolated from the first two funnels (8.6 g.) was redistributed in the first buffer system employed, yielding 4.3 g. of residue from the first two funnels. This material was combined with the 5.1 g. of substance isolated from the first distribution and was chromatographed on basic alumina, activity IV (38 × 200 mm.), eluting with increasing amounts of methanol in methylene chloride: 1% methanol (1.6 l.), 0.94 g.; 5% methanol (1.2 l.), 1.83 g.; 8% methanol (250 ml.), 0.24 g.; 10% methanol (500 ml.), 0.18 g.; 14% methanol (800 ml.), 0.72 g.; 20% methanol (1.3 l.), 0.51 g. The 8% and 14% methanol fractions crystallized from ethanol yielding neburnamine (400 mg.), m.p. 285–290°, increasing to 290–292° on recrystallizing from methylene chloride; $[\alpha]_D$ $-199°$ (methanol); pK_a' 9.9, 7.7; $\lambda_{\max}$ ($\epsilon_{1\,\text{cm}}^{1\%}$) 293 (140); λ_{sh} 230 (460), 284 (130); $\lambda_{\min}$ 262 (52); $\lambda_{\max}^{\text{acid}}$ 244 (119), 293 (143); λ_{sh} 274 (128), 284 (130); $\lambda_{\min}$ 262 (70); $\nu_{OH|NH}$ 3600, 3460, 3250; ν_{CO} 1741.

Anal. Found: C, 62.70, 63.30, 63.30, 66.16, 67.60; H, 7.24, 7.42, 7.45, 7.48, 7.63; N, 7.10, 7.32, 7.48; NCH_3, 7.58; OCH_3, 3.73.

The Methylene Chloride-Soluble Bases.—The crude methylene chloride extracted bases (119 g.) were chromatographed on basic alumina, activity III (100 × 480 mm.) with the following fractions accumulating: methylene chloride (750 ml.), 6.0 g.; methylene chloride (4 l.); 72.3 g.; methanol–methylene chloride (3 l., 10%), 30 g. The second methylene chloride fraction (72.3 g.) was rechromatographed on alumina (100 × 150 mm.) with the eluates: benzene–cyclohexane (4 l., 1:1), 4.4 g.; benzene–cyclohexane (8 l., 1:1) 12.8 g.; benzene (2 l.), 3.2 g.; benzene (6 l.), 6.6 g.; benzene (9 l.), 2.6 g.; methylene chloride (2 l.), 1.7 g.

Eburnamonine (**III**).—The first methylene chloride eluate (6.0 g.) of the first chromatogram and the second benzene–cyclohexane eluate (12.8 g.) of the second chromatogram gave from ethanol 1.37 g. and 10.5 g. of eburnamonine, respectively, m.p. 183°; pK_a' 6.1; $[\alpha]_D$ $+89°$, $\lambda_{\max}$ 241 (19,800), 268 (10,200), 295 (4800), 303 (4800); $\lambda_{\min}$ 220 (8400), 258 (9400), 288 (4200), 299 (4600); ν_{CO} 1700.

Anal. Calcd. for $C_{19}H_{22}N_2O$: C, 77.52; H, 7.53; N, 9.52. Found: C, 77.79; H, 7.46; H, 9.48.

Pleiocarpine (V, R_1 = $COOCH_3$, R_2 = H_2).—The first benzene eluate (3.2 g.) of the second chromatogram gave pleiocarpine (92 mg.), m.p. 139–149°, increasing to 144–145°, from ethanol–water; $[\alpha]_D$ $-141°$; pK_a' 7.3; $\lambda_{\max}$ 245 (13,800), 281 (3000), 289 (2700); $\lambda_{\min}$ 224 (6000), 267 (1800), 287 (2700); ν_{CO} 1724, 1705–1710. For analysis one sample was dried at 78° *in vacuo* and another sublimed at 140–147° (0.01 mm.).

Anal. Calcd. for $C_{23}H_{28}N_2O_4$: C, 69.67; H, 7.12; N, 7.07. Found: C, 69.64, 69.29; H, 6.97, 6.95; N, 7.26, 7.16.

Pleiocarpamine.—The second benzene eluate (6.6 g.) of the second chromatogram gave from ethanol–water pleiocarpamine (2.1 g.), m.p. 152–155°, increasing to 164–165° upon recrystallization from ether; $[\alpha]_D$ $+ 123°$; pK_a' 7.3; $\lambda_{\max}$ 230 (29,600), 285 (8200); λ_{sh} 294 (6700); $\lambda_{\min}$ 253 (3300); ν_{CO} 1730 (ester); $\nu_{CO}^{CHCl_3}$ 1761, 1730.

Anal. Calcd. for $C_{20}H_{22}N_2O_2$: C, 74.51; H, 6.88; N, 8.69; OCH_3, 9.64. Found: C, 74.28, 74.57; H, 6.98, 7.03; N, 8.80, 8.68; OCH_3, 9.12.

Although it could be sublimed at 160–180° *in vacuo,* it slowly decomposed at 100° under otherwise similar conditions.

Eburnamenine (IV).—The first fraction (2.5 g.) of the second chromatogram was rechromatographed on basic activity III alumina (38 × 130 mm.) eluting eburnamenine (3.6 g.) with 1:1 hexane–benzene, characterized as its picrate (4.3 g.), m.p. 186–187°, increasing to 196° from ethanol.

Anal. Calcd. for $C_{25}H_{25}N_5O_7$: C, 59.16; H, 4.97; N, 13.80. Found: C, 59.30; H, 5.09; N, 12.67.

A sample of the picrate was decomposed by filtration through alumina in methylene chloride yielding eburnamenine, which was sublimed at 140° (0.01 mm.) for analysis; $[\alpha]_D$ $+183°$; pK_a' 6.45; $\lambda_{\max}$ 223 (23,200), 258 (29,200), 301 (7600), 311 (8600); $\lambda_{\min}$ 236 (12,000), 285 (5900), 305 (6300).

Anal. Calcd. for $C_{19}H_{22}N_2$: N, 10.06. Found: N, 9.88.

Acknowledgment.—We wish to thank Dr. E. Schlittler for his constant interest and encouragement. We are indebted to Mr. L. Dorfman and his staff for the analytical results, to Dr. A. F. Zürcher for the p.m.r. spectra, and to Dr. C. Rehm and his staff for the potentiometric microtitrations.