[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

Aspidospermine. I

BY BERNHARD WITKOP

Aspidospermine, $C_{22}H_{30}O_2N_2$, isolated from the bark of *Aspidosperma quebracho*[1,2] and from the leaves of *Vallesia glabra*,[3,4] was last studied by Ewins.[5] He found the alkaloid stable toward catalytic hydrogenation, and to contain an N-acetyl group, a methoxyl group attached to an aromatic ring, and possibly a reduced quinoline nucleus. Schlittler and Rottenberg[6] very recently described an alkaloid from *Vallesia glabra* which apparently is the N-formyl analog of aspidospermine (formyldesacetylaspidospermine).

In order to classify aspidospermine, dehydrogenation with zinc and palladium was employed. The former treatment yielded two major fragments. The non-basic moiety, an indole derivative, proved very difficult to purify, a fact which suggests the presence of a mixture of isomorphous homologs inseparable by crystallization. In the cases of yohimbine[7] and C-dihydrotoxiferine[8] these homologs were skatol and 3-ethylindole. Since all indole alkaloids show a clear relation to tryptophan, it is very likely that similar conditions obtain also in the case of aspidospermine. The structure of this part of the molecule will be resumed in a later investigation.

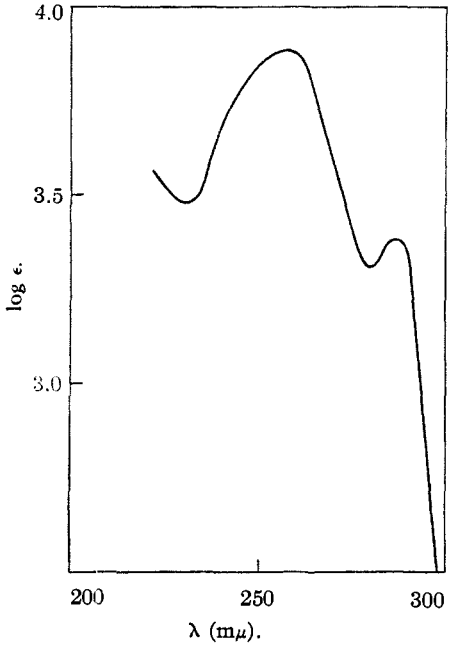


Fig. 1.—Ultraviolet absorption spectrum of aspidospermine (in ethanol).

The ultraviolet absorption spectrum of aspidospermine (Fig. 1) suggests the presence of a dihydroindole structure[8a]; similarly the action of ozone[7] results only in the formation of an amine oxide ("genaspidospermine") which on hydrolysis is converted to desacetylaspidospermine N-oxide, on thermal decomposition to aspidospermine.

Desacetylaspidospermine exhibits the same red color reaction with concentrated nitric acid (brucinol reaction) which has earlier been demonstrated for geissospermine (containing one methoxy group)[9] and for toxiferine[8] (containing no methoxy group). The latter two alkaloids, also belonging to the indole group, yield 3-ethylpyridine on dehydrogenation.

In the case of aspidospermine, the basic fraction, resulting from dehydrogenation, a colorless volatile oil of characteristic odor, was isolated as the beautifully crystalline picrate. The compound was not identical with bz-tetrahydroisoquinoline.[10,11] The exact analysis, $C_9H_{13}N$, the ultraviolet absorption spectrum (Fig. 2) and the infrared spectrum (Fig. 3) suggested a pyridine homolog. Since a relationship between aspidospermine and quinamine[12] was suspected, 3,4-diethyl- and 2,4-dimethyl - 3 - ethylpyridine (VI), possibly derivable from quinuclidine structures I and II by dehydrogenation, were at first considered. The former parvoline was ruled out by the much lower melting point of the picrate,[13] whereas the latter synthe-

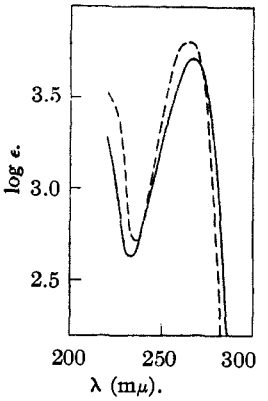
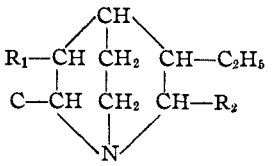
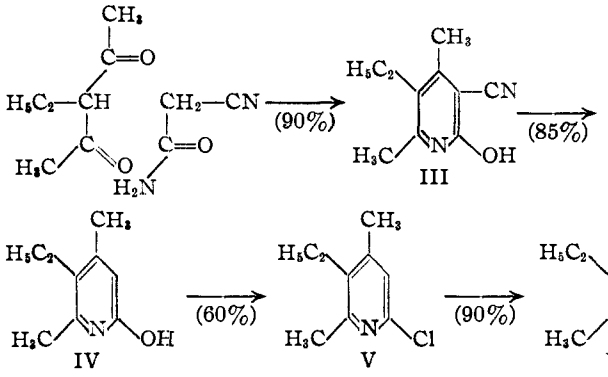


Fig. 2.—3,5-Diethylpyridine hydrochloride, — (from aspidospermine); 2,4 - dimethyl - 3 - ethylpyridine hydrochloride, ------ (solvent ethanol).



I, $R_1 = CH_3$, $R_2 = H$
II, $R_1 = H$; $R_2 = CH_3$

(1) Fraude, *Ber.*, **11**, 2189 (1878); **12**, 1560 (1879).
(2) Hesse, *Ann.*, **211**, 249 (1882).
(3) Hartmann and Schlittler, *Helv. chim. acta*, **22**, 547 (1939).
(4) Deulofeu, *et al.*, *J. Chem. Soc.*, 1051 (1940).
(5) Ewins, *ibid.*, **105**, 2738 (1914).
(6) Schlittler and Rottenberg, *Helv. Chim. Acta*, **31**, 446 (1948).
(7) Witkop, *Ann.*, **556**, 103 (1944).
(8) Wieland, Witkop and Bähr, *ibid.*, **558**, 144 (1947).
(8a) *Cf.* Raymond-Hamet, *Compt. rend.*, **226**, 2154 (1948); Openshaw and Smith, *Experientia*, in press.
(9) Bertho and Sarx, *Ann.*, **556**, 22 (1944).
(10) Schlittler and Merian, *Helv. Chim. Acta*, **30**, 1339 (1947).
(11) Witkop, THIS JOURNAL, **70**, 2617 (1948).
(12) Kirby, *J. Chem. Soc.*, 528 (1945).
(13) Koenigs and Bernhart, *Ber.*, **38**, 3049 (1905).



sized by conventional methods[14] (III–VI), gave quite dissimilar physical constants of its salts (Table I) as well as a deviating infrared spectrum (Fig. 5).

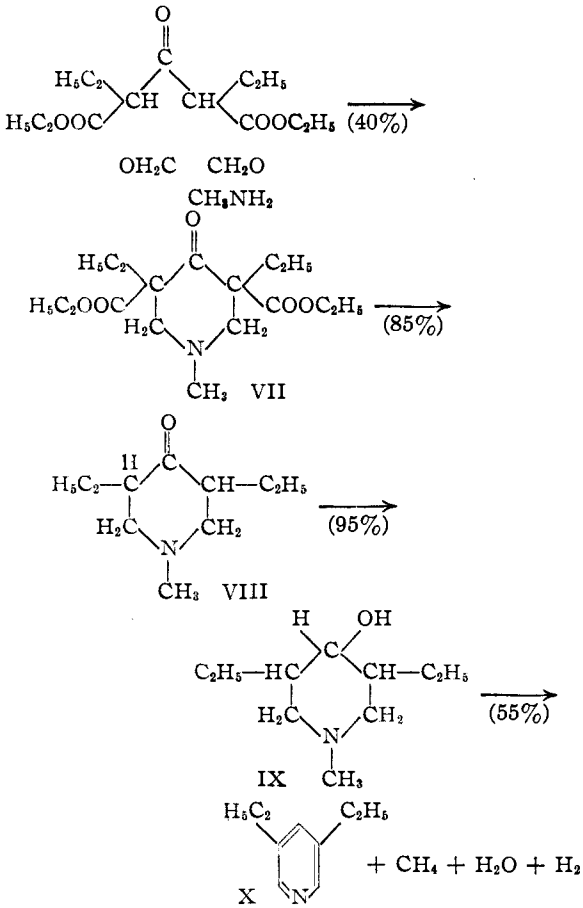
TABLE I

	Hydrochloride	Chloroaurate	Chloroplatinate	Picrate
2,4-Dimethyl-3-ethylpyridine	189–191	137	223–225	137–139[15]
3,5-Diethylpyridine	169–172	213	245 (196)[a]	159
$C_9H_{13}N$ from aspidospermine	169–172	203	245 (196)[a]	179–182
3,5-Dimethylpyridine		146–147	254–255[16]	245[17]

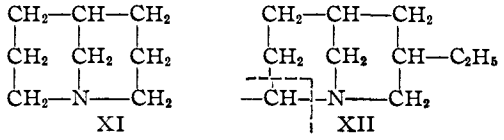
[a] Very characteristic melting, resolidification and renewed melting.

The unusually high melting point of the unknown picrate then suggested a symmetrically substituted pyridine,[17a] since 3,5-dimethylpyridine picrate has a melting point of 245°.[17] Tschitschibabin has prepared two further symmetrical parvolines, 3,5-dimethyl-4-ethylpyridine (chloroaurate, m. p. 138–140°, chloroplatinate melting above 270°[18]) and 3,5-diethylpyridine.[19] Since the latter lacked any characterization, it was synthesized, by an adaptation of Prelog's β-collidine synthesis,[20] from 3,5-diethyl-4-piperidinol (IX). This alkamine was obtained by reduction with lithium aluminum hydride of (VIII), the decarboxylated condensation product of a Mannich reaction between diethyl α,α′-diethylacetonedicarboxylate, formaldehyde and methylamine.[21]

The physical constants (Table I) and the infrared absorption spectra (Figs. 3 and 4) show the far-reaching identity between the parvoline from aspidospermine and the synthetic base. However, some discrepancies are notable. A difference occurs between the melting points of the picrates and the chloroaurates of the unknown pyridine and the compound synthesized for comparison. This is probably due to partial breakdown of the side chains in the unknown base at the high temperature of the zinc dust distillation and mixed crystal formation between the picrate and the chloroaurate of 3,5-diethylpyridine and small amounts of the corresponding picrate and chloroaurate of 3,5-dimethylpyridine, respectively (*v. s.* the analogous case of 3-ethylindole-skatol). Further support of this explanation is rendered by the absence of depression on admixture and the higher rather than lower melting point of the picrate from aspidospermine.



Although Prelog and Balenovic[22] were unable to obtain a crystalline picrate from the oily dehydrogenation products of 1-aza-bicyclo(1,3,3)-



(14) Bardhan, *J. Chem. Soc.*, 2231 (1929).

(15) In this connection, it is interesting to recall that Barger, Dyer and Sargent, *J. Org. Chem.*, **4**, 418 (1939), obtained a similar pyridine picrate (m. p. 134–135°) from rotundifoline by selenium dehydrogenation. By courtesy of Drs. Dyer and Sargent, who kindly placed at my disposal a small amount of the original picrate, the two picrates could be compared directly. The depression (115–120°) observed on admixture excludes identity.

(16) Ahrens and Gorkow, *Ber.*, **37**, 2062 (1904); Eckert and Loria, *Monatsh.*, **38**, 225 (1917).

(17) Bratton and Bailey, THIS JOURNAL, **59**, 175 (1937).

(17a) The possible significance of the high melting point of the picrate was first pointed out by Dr. R. B. Woodward.

(18) *Cf.* Beilstein, IV, **20**, 254.

(19) Tschitschibabin, *J. prakt. Chem.*, **107**, 126 (1923).

(20) Prelog, *Ber.*, **74**, 1705 (1941).

(21) Mannich and Schumann, *ibid.*, **69**, 2301 (1936).

(22) Prelog and Balenovic, *ibid.*, **74**, 1508 (1941).

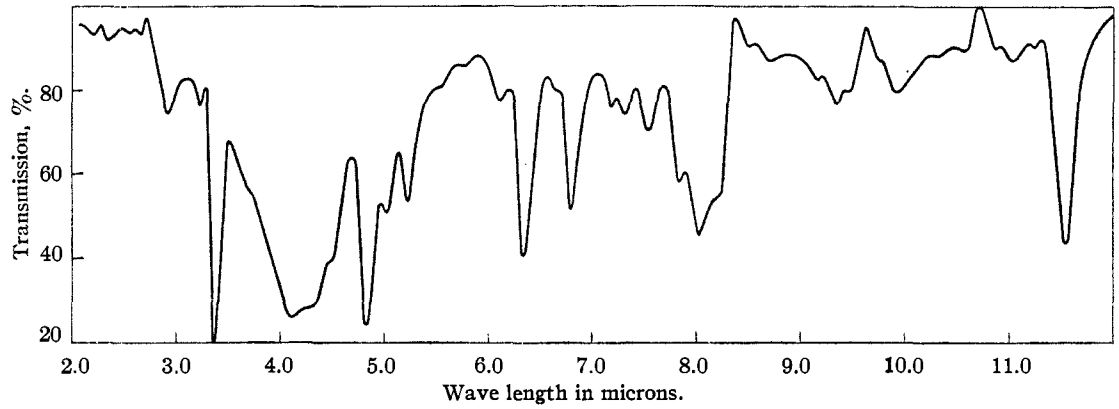


Fig. 3.—3,5-Diethylpyridine hydrochloride (from aspidospermine) in chloroform.

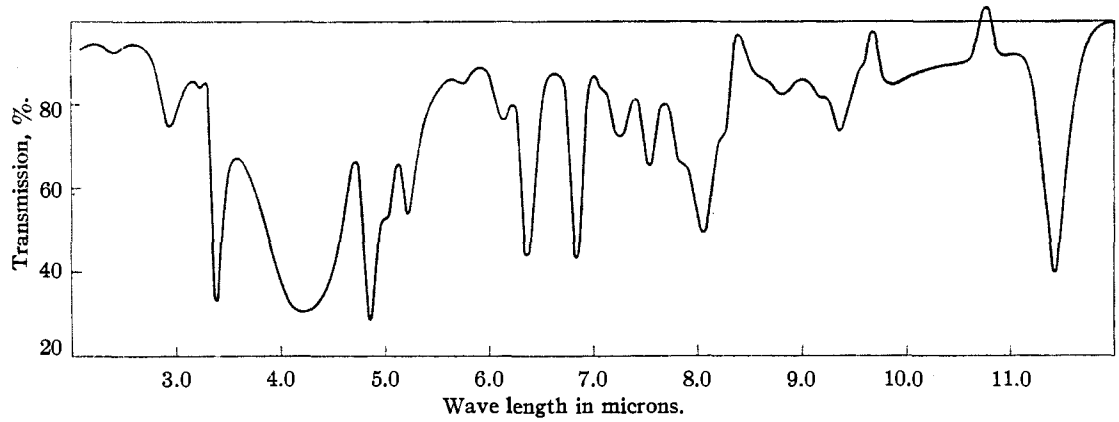


Fig. 4.—3,5-Diethylpyridine hydrochloride (synthetic) in chloroform.

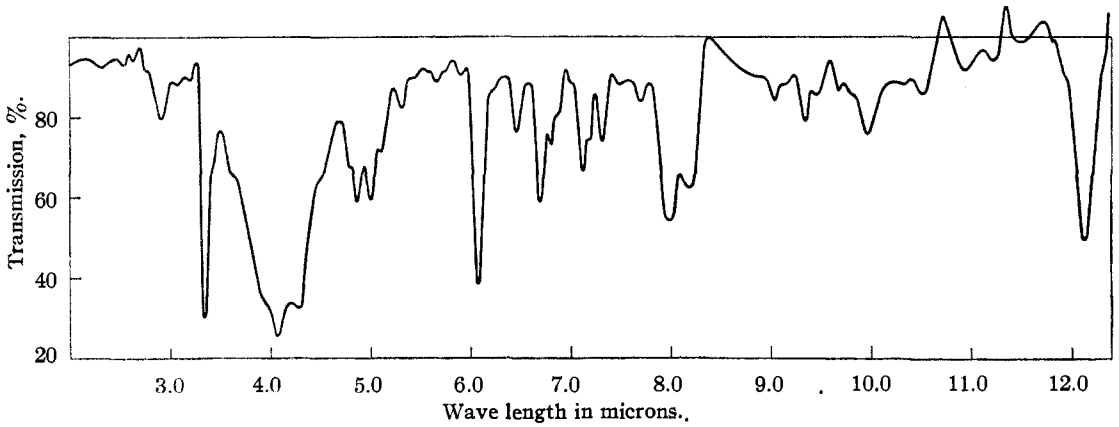


Fig. 5.—2,4-Dimethyl-3-ethylpyridine hydrochloride in chloroform.

nonane (XI)[23] when treating the latter with selenium for thirty hours at 350°, it is possible that zinc dust distillation of (XII) may lead to 3,5-diethylpyridine.

The cleavage of aspidosine (desacetyldesmethylaspidospermine) in the zinc dust distillation can be formulated

$$C_{19}H_{26}ON_2 \longrightarrow C_{10}H_{11}N + C_9H_{15}N$$

(23) Prelog, Heimbach and Seiwerth, *Ber.*, **72**, 1319 (1939).

Physiological Activity of Aspidospermine.—The antimitotic activity was kindly investigated by H. Lettré[24] who found that aspidospermine has a distinct inhibitory effect on growth and mitosis of chicken heart fibroblasts (70–140 γ/cc. of tissue culture fluid) which is, however, very much weaker than that of colchicine (0.05 γ/cc.).

(24) Private communication.

Experimental[25]

Zinc Dust Distillation of Desacetylaspidospermine Hydrochloride.—Preliminary experiments showed that aspidospermine itself survives in the zinc dust distillation to an extent of 60%. The use of the desacetyl base gave better results; to decrease the volatility of the latter (m. p. 110°), the hydrochloride was employed (prepared from purest aspidospermine). For every distillation 50 mg. of hydrochloride was mixed with 5 g. of zinc dust (reagent). The reaction was carried out in an apparatus described previously.[26] Eighty such distillations were carried out.

Basic Fraction.—The combined ethereal solutions of the volatile degradation products were concentrated to 50 cc. and extracted twice with 5 cc. of dilute alkali. From this alkaline solution, after acidification and extraction with ether, a very small amount of a phenolic compound was obtained bearing no substituent in the para position (*cf.* reference 26). The ether solution was then extracted several times with 2 *N* hydrochloric acid. The bases were liberated with alkali and taken up in ether. The volatile bases were separated from the dark colored basic residue by steam distillation. The volatile base, characterized by a strong and peculiar odor (reminding of horse urine), was obtained from the steam distillate by salting out with ammonium chloride and extraction with ether. Renewed distillation *in vacuo* gave a constant boiling colorless oil (110° (10 mm.)), not miscible with water but perceptibly soluble in it. The base was dissolved in 2 cc. of ether and extracted with consecutive portions of 0.5 cc. of 0.1 *N* hydrochloric acid. By addition of aqueous picric acid solution the following picrates were obtained.

Picrate A: this picrate deposited from the first fraction in long needles after twelve hours, melting point 155–158°, after recrystallization from methanol 168–172°. On admixture with picrate B or with synthetic 3,5-diethylpyridine picrate a large depression (140°) was observed. The paucity of this picrate did not allow of further investigation.

Picrate B: all the subsequent fractions gave directly crystalline picrates that proved to be uniform; melting point after recrystallization from little acetone, 180–182° (sintering at 168°). The picrate formed uniform, beautiful glossy needles.

Anal. Calcd. for $C_9H_{13}N \cdot C_6H_3O_7N_3$: C, 49.44; H, 4.39. Found: C, 49.51; H, 4.18.

A mixture of equal parts of this picrate and synthetic 3,5-diethylpyridine picrate (m. p. 159°) sintered at 168° and melted at 182°. A mixture of this picrate and the higher melting synthetic picrate (170–174°) showed the same behavior.

Hydrochloride.—Purest picrate B (30 mg.) was triturated with 2 cc. of 4 *N* hydrochloric acid and extracted frequently with ether until the latter was colorless. After evaporation to dryness in the desiccator the hydrochloride was obtained in fine crystals which, after recrystallization from alcohol/ether (1:4), melted at 169–172°.

Chloroplatinate.—When a few drops of a solution of platinum chloride in 2 *N* hydrochloric acid were added to a solution of 2 mg. of the above hydrochloride in 0.3 cc. of water, yellow-red plates deposited after scratching and standing. Dried over phosphorus pentoxide, the crystals melted at 196°, resolidified above 200° and formed a dark melt at 243–245°.

Anal. Calcd. for $(C_9H_{13}N)_2 \cdot H_2PtCl_6$: C, 31.76; H, 4.15; Pt, 28.7. Found: C, 31.65; H, 4.23; Pt, 28.3.

Chloroaurate.—The addition of gold chloride to a solution of the hydrochloride produced an immediate precipitate of bright yellow fine needles; melting point, after two recrystallizations from methanol containing a drop of water, 201–203°. This melting point was not lowered on admixture with synthetic 3,5-diethylpyridine chloroaurate (m. p. 213°).

The dark residue of the steam distillation was distilled *in vacuo;* at 160° (0.01 mm.) one obtained a colorless oil which, on treatment with methyl iodide, gave glistening needles showing, after recrystallization from methanol, the melting point of desacetylaspidospermine dimethiodide, 176–177°.[5] Some of the untreated base was converted into the picrate which finally crystallized from methanol in plates; like desacetylaspidospermine picrate it softened between 120–130°.

Non-Basic Fraction.—The ethereal solution, which was now free of bases, was evaporated. The residue was separated by steam distillation into a steam volatile and a non-volatile part. The volatile part, which had a strong indolic odor, was distilled in high vacuum. The colorless oil which distilled at 80–90° was dissolved in a few drops of benzene. On addition of a cold solution of picric acid in benzene and not too much petroleum ether one obtained a red picrate. After four recrystallizations (benzene/petroleum ether, finally benzene) the picrate formed clusters of dark red needles, transformation point 136°, sintering 148°, melting 156°.

Anal. Calcd. for $C_{10}H_{11}N \cdot C_6H_3O_7N_3$: C, 51.33; H, 3.74. Found: C, 53.02; H, 4.30. N-Methyl determination: 2.61% "NCH_3" (*cf.* ref. 26, footnote 23).

Infrared Spectra.—3-Ethylindole (measured in chloroform and carbon disulfide) shows the following main bands: 2.84; 3.4; 6.84; 7.45; 9.16; 13.5μ. The mixture of the crude steam-volatile indoles shows a somewhat weaker NH band (2.84), a slightly stronger CH band (3.4). The bands at 6.83 and 13.5 are present in about the same strength. The very strong band at 13.5 is also present in skatol and, therefore, cannot be a shifted C-ethyl band (12.8).

The residue from the steam distillation could be purified by high vacuum distillation. One obtained prisms from petroleum ether, melting point 135°. Further purification was not possible. The Hopkins–Cole reaction was negative, the Ehrlich reaction crimson already in the cold.

Aspidospermine and Ozone.—Aspidospermine (1.5 g.) was dissolved in a mixture of 10 cc. of glacial acetic acid and 5 cc. of water. The solution was treated with an excess of 4% ozone. The faintly yellow solution was diluted with water and made slightly alkaline (pH 8). The precipitated base (1.2 g.) was unchanged aspidospermine (m. p. 208°). When the slightly alkaline mother liquor was made strongly alkaline by the addition of excess 50% potassium hydroxide, a colorless base (0.25 g.) deposited which, after recrystallization from absolute ethanol, formed fine needles, melting point 183°.

Anal. Calcd. for $C_{22}H_{30}O_3N_2$: C, 71.35; H, 8.10; N, 7.58. Found: C, 71.32; H, 7.97; N, 7.49.

Genaspidospermine possesses a stability that is somewhat unusual for an amine oxide: (i) aqueous sulfurous acid (ten hours, 20°) fails to reduce the oxide, (ii) high vacuum distillation (150°) does not decompose it. A solution of genaspidospermine in dilute acid remains clear on the addition of ammonia; this makes the separation from aspidospermine very easy.

Reconversion of Genaspidospermine into Aspidospermine.—At a pressure of 0.5 mm. and a temperature of 240°, 20 mg. of genaspidospermine sublimed in colorless fine needles without leaving any residue. When these needles were dissolved in dilute acid and ammonia was added, a base was obtained which, after recrystallization from ether, melted at 208° and showed no depression with genuine aspidospermine.

Desacetylgenaspidospermine.—Genaspidospermine, on boiling with 10% hydrochloric acid for two hours, lost its acetyl group. The solution was made strongly alkaline and extracted with ether. The base obtained from the ether extract was purified by distillation in high vacuum (120°) and by recrystallization from low boiling petroleum ether; melting point 163° (sintering 148°).

Anal. Calcd. for $C_{20}H_{28}O_2N_2$: C, 73.17; H, 8.53; OCH_3, 9.45. Found: C, 73.26; H, 8.27; OCH_3, 8.68.

4,6-Dimethyl-5-ethyl-2-pyridone (IV).—When 12.5 g. of 3-cyano-4,6-dimethyl-5-ethyl-2-pyridone (III)[14] (melting point 282–284°) were boiled for three hours with 50 cc.

(25) All melting points are corrected.

(26) *Cf.* Witkop, THIS JOURNAL, **70**, 1424 (1948).

of 80% sulfuric acid saponification and decarboxylation occurred simultaneously.[27] The cool solution was diluted, neutralized with alkali and acidified with acetic acid. The pyridone (11.2 g.) crystallized in colorless needles; melting point, after recrystallization from water or little alcohol, 192° (sublimes).[28]

Anal. Calcd. for $C_9H_{13}ON$: C, 71.52; H, 8.66. Found: C, 71.72; H, 8.51.

2-Chloro-4,6-dimethyl-5-ethylpyridine (V).—The dry pyridone (16 g.) was moistened with 15 cc. of phosphorus oxychloride. The mixture was heated to 100–120° and 25 g. of phosphorus pentachloride was added gradually during the course of twenty minutes. The bath temperature was then raised to 140° and held at that point for forty-five minutes. After cooling the phosphorus oxychloride was removed under reduced pressure, the residue was poured onto cracked ice, and the solution was made strongly alkaline with sodium hydroxide. The chloropyridine was steam distilled from this alkaline solution and extracted from the distillate with ether. On distillation it boiled at 106° (5 mm.). The yield was 11 g.

Anal. Calcd. for $C_9H_{12}NCl$: C, 64.43; H, 7.23. Found: C, 64.48; H, 7.23.

Picrate.—From the warm solution of the chloropyridine in mineral acid picric acid precipitated the oily picrate which became crystalline on scratching and standing, melting point 88°.

Anal. Calcd. for $C_9H_{12}NCl \cdot C_6H_3O_7N_3$: C, 45.33; H, 4.5. Found: C, 45.26; H, 4.32.

2,4-Dimethyl-3-ethylpyridine (VI).—When 3.8 g. of the chloropyridine in 15 cc. of alcohol was hydrogenated in the presence of 0.1 g. of active palladium black, 565 cc. of hydrogen (calcd. 520 cc.) was absorbed in the course of six hours. The alcoholic solution, after separation from the palladium and evaporation, gave directly the crystalline pyridine hydrochloride (3.6 g.). Recrystallized from alcohol/ether, it melted at 189–191° (subl.).

Anal. Calcd. for $C_9H_{13}N \cdot HCl$: C, 62.96; H, 8.16. Found: C, 63.17; H, 8.40.

Picrate.—The picrate, prepared from the aqueous solution of the hydrochloride, was recrystallized from acetone, melting point 137–139°.

Anal. Calcd. for $C_9H_{13}N \cdot C_6H_3O_7N_3$: C, 49.44; H, 4.39. Found: C, 49.60; H, 4.38.

Chloroplatinate.—Orange yellow rectangular prisms from water, easily soluble in warm water, melting point 223–225°.

Anal. Calcd. for $(C_9H_{13}N)_2 \cdot H_2PtCl_6$: C, 31.76; H, 4.15; Pt, 28.7. Found: C, 32.10; H, 4.22; Pt, 29.06.

Chloroaurate.—Bright yellow prisms from water, melting point, after recrystallization from methanol/water, 137° (sintering 125°).

Anal. Calcd. for $C_9H_{13}N \cdot HAuCl_4$: C, 22.8; H, 2.96; Au, 41.7. Found: C, 23.14; H, 2.96; Au, 42.09.

α-N-Methyl-3,5-diethyl-4-piperidinol (IX).—When 5.5 g. of N-methyl-3,5-diethyl-4-piperidone (VIII)[21] in 50 cc. of absolute ether was added to an excess of lithium aluminum hydride in absolute ether in the course of thirty minutes, vigorous reduction took place. After decomposition of the reaction mixture with ice and strong alkali, the ether extract gave 5.2 g. of colorless crystals melting, after recrystallization from petroleum ether, at 105–107°.

Anal. Calcd. for $C_{10}H_{21}ON$: C, 70.17; H, 12.26. Found: C, 69.65; H, 12.35.

Hydrochloride.—When dry hydrogen chloride was passed through a solution of the piperidinol in absolute ether, the crystalline hydrochloride separated, melting point, after recrystallization from absolute alcohol, 225°. The salt is slightly hygroscopic.

3,5-Diethylpyridine Hydrochloride (X).—For dehydrogenation 0.5-g. portions of the above hydrochloride were heated with 0.2 g. of palladium black to 280° for one to two hours. The water formed in the reaction could evade and condense in a trap. When there was no more water formed, the reaction was stopped; 83 cc. of hydrogen and methane were formed. After distillation (90° (5 mm.)) the colorless pyridine (0.15 g.) was dissolved in absolute ether and converted into the crystalline hydrochloride by passing dry hydrogen chloride through the solution. From alcohol ether (1:4) the hydrochloride crystallized in beautiful long needles, melting point 169–172° (subl.).

Anal. Calcd. for $C_9H_{13}N \cdot HCl$: C, 62.96; H, 8.16. Found: C, 62.56; H, 8.26.

Picrate.—The picrate was recrystallized several times from methanol and acetone; it formed uniform long needles, melting point 159° (analysis I); repeated recrystallizations from acetone gave a small fraction of a higher melting picrate, m. p. 170–174° (analysis II).

Anal. Calcd. for $C_9H_{13}N \cdot C_6H_3O_7N_3$: C, 49.44; H, 4.39. Found: C, 49.72; H, 4.18 (analysis I); C, 49.53; H, 4.24 (analysis II).

Chloroplatinate.—The addition of platinum chloride to a solution of 3,5-diethylpyridine hydrochloride in hydrochloric acid did not directly produce a precipitate. After standing in the cold and scratching yellow-reddish platelets deposited, melting at 196–200°, resolidifying above 200°, melting a second time under decomposition at 245°.

Anal. Calcd. for $(C_9H_{13}N)_2 \cdot H_2PtCl_6$: C, 31.76; H, 4.15; Pt, 28.7. Found: C, 31.89; H, 4.16; Pt, 27.9.

Chloroaurate.—The oily chloroaurate produced in aqueous solution became crystalline on standing. Recrystallized from methanol, it formed fine yellow needles, melting point 213–216°.

Methiodide.—3,5-Diethylpyridine hydrochloride was dissolved in acetone and left overnight with excess methyl iodide. After evaporation to dryness the residue was recrystallized from alcohol/ether yielding prisms, melting point 143° (sintering 135°).

Anal. Calcd. for $C_9H_{13}N \cdot CH_3I \cdot 0.5H_2O$: C, 41.95; H, 5.94. Found: C, 41.68; H, 5.64.

Methyl Picrate.—On addition of aqueous picric acid to a solution of the above methiodide in water the picrate separated in bright yellow scales, melting point 139–141°.

Anal. Calcd. for $C_{10}H_{16}N \cdot C_6H_3O_7N_3$: C, 50.65; H, 5.01. Found: C, 50.97; H, 4.86.

Acknowledgment.—The author is indebted for support of this work to Prof. L. F. Fieser in whose laboratory part of this work was performed. The ultraviolet spectra were kindly measured by Miss Adelaide Sutton through the courtesy of Dr. Elkan R. Blout (Polaroid Corporation, Cambridge).

Summary

Aspidospermine, a new type of indole alkaloid, was degraded to 3,5-diethylpyridine and an alkyl indole. The former and 2,4-dimethyl-3-ethylpyridine were synthesized in the course of this investigation.

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(27) Bardhan[14] states that hydrolysis of the nitrile can be effected by concentrated hydrochloric acid. In this investigation it was noted that even boiling for fifteen hours with 48% hydrobromic acid leaves some of the nitrile unchanged. This steric hindrance in the saponification reaction is not observed in nitriles lacking the neighboring methyl group, Tracy and Elderfield, *J. Org. Chem.*, **6**, 63 (1941).

(28) Bardhan reports a melting point of 150° for the pyridone.