[JOINT CONTRIBUTION FROM THE FACULTAD DE QUIMICA Y FARMACIA, UNIVERSIDAD NACIONAL DE LA PLATA AND THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY]

Isolation of Quebrachamine and of a New Dihydroindole Alkaloid, Spegazzinine, from *Aspidosperma chakensis* Spegazzini[1,2]

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From the bark of *Aspidosperma chakensis* Spegazzini there have been isolated quebrachamine and a new alkaloid, now named spegazzinine ($C_{21-22}H_{28-30}N_2O_3$). The presence of a N-acetyldihydroindole nucleus, a phenolic and an alcoholic hydroxyl group has been established and evidence for the location of the phenolic substituent is presented. Spegazzinine may be a hydroxylated demethylaspidospermine.

Chemical investigations of alkaloids from various *Aspidosperma* and *Vallesia* species have been carried out for some time[5] but the constitutions of the principal alkaloids, quebrachamine ($C_{19}H_{26}N_2$) and aspidospermine ($C_{22}H_{30}N_2O_2$) are as yet unknown although working structures have been proposed[6,7] for the latter. The great current interest in *Apocynaceae* alkaloids, particularly of the genus *Rauwolfia*, has prompted us to examine other new species of this plant family. We should now like to report our results with the Argentinian *Aspidosperma chakensis* Spegazzini[8] which grows in the Chaco where it is referred to by the natives as "ubirá-ro-puiita" and employed as a febrifuge, as a remedy for snake bites, etc.

The bark samples[9] were put at our disposal through the kind cooperation of Prof. José F. Molfino (Instituto de Botanica y Farmacologia de la Facultad de Ciencias Medicas, Universidad de Buenos Aires) who also established the botanical authenticity by comparison with herbarium specimens. The crude alkaloids were processed by a scheme described in detail in the experimental section, fractionation being accomplished most readily by taking advantage of the differential solubility of the oxalates. From the soluble oxalate fraction after removal of phenolic material there was isolated approximately 0.01% of a crystalline alkaloid which was shown to be identical with quebrachamine[10,11] by mixture melting point determination, infrared comparison, and virtual coincidence of the rotatory dispersion curves over the range 700–320 mμ. The identity of the two alkaloids was further confirmed by direct comparison of the respective methiodides and picrates.

The principal alkaloid (*ca.* 0.6% based on dry bark) proved to be phenolic and could be separated readily as the insoluble oxalate; we have named it "spegazzinine" after the famous Argentinian botanist who first described this species.[8] Spegazzinine is a beautifully crystalline base but the tenacious retention of solvent by the alkaloid and its crystalline salts as well as the non-crystalline nature of most of its transformation products preclude a firm assignment of its empirical formula. The majority of the analytical results are compatible with a $C_{21}H_{28}N_2O_3$ formulation, which is also favored because of its presumed relationship (*vide infra*) with demethylaspidospermine[6] ($C_{21}H_{28}N_2O_2$), but $C_{22}H_{30}N_2O_3$ cannot be excluded definitely at this time. The ultraviolet absorption spectrum (Fig. 1) is very similar to that of aspidospermine[6] and the infrared spectrum (Fig. 2) clearly shows the presence of an amide grouping. Functional group analysis demonstrated the presence of one C-methyl and one acetyl group and the absence of methoxyl and probably[12] also N-methyl groups. The alkaloid was further characterized as the crystalline hydrochloride and oxalate (monohydrate) salts.

(1) Paper XIII in the Wayne series "Alkaloid Studies" [preceding paper, Djerassi, Markley, and Ehrlich, *J. Org. Chem.*, **21**, 975 (1956)] and paper III in the series "Estudios Sobre Plantas" (preceding paper, ref. 5a).

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(5) For leading references see (a) Orazi, *Anal. Asoc. Quim. Argentina*, **34**, 158 (1946); (b) Henry *The Plant Alkaloids*, Blakiston Co., Philadelphia, 1949, 4th edit., pp. 511–515; (c) Marion in Manske and Holmes *The Alkaloids*, Academic Press, New York, 1952, Vol. II, pp. 422–424.

(6) Witkop and Patrick, *J. Am. Chem. Soc.*, **76**, 5603 (1954); Witkop, *J. Am. Chem. Soc.*, **70**, 3712 (1948).

(7) Openshaw quoted by Robinson in *Structural Relations of Natural Products*, Oxford University Press, 1955, p. 118.

(8) Spegazzini, *Physis* (*Buenos Aires*), **3**, 333 (1917).

(9) Collected in January 1949 in the Chaco between Tres Isletas and Saénz Peña by Ing. Agr. Arturo E. Ragonese and Prof. Bruno I. Piccinini.

(10) Field, *J. Chem. Soc.*, 1444 (1924).

(11) We are greatly indebted to Dr. B. Witkop (National Institutes of Health, Bethesda) and Dr. Walter B. Mors (Instituto de Quimica Agricola, Rio de Janeiro) for authentic specimens.

(12) In four instances, a low N-methyl value was observed but this also proved to be the case in a parallel determination carried out with aspidospermine, which is reported not to contain such a grouping [Ewins, *J. Chem. Soc.*, 2738 (1914) and ref. 13]. Witkop [*J. Am. Chem. Soc.* **71**, 2559 (1949)] has shown that abnormally high blank values for N-methyl can be obtained in this series of alkaloids.

Acid cleavage of spegazzinine (I) produced crystalline deacetylspegazzinine (II), which did not show any more the amide band in the infrared (Fig. 2) thus demonstrating the presence of an N-acetyl moiety in I. The nature of the remaining two oxygen atoms was established in the following manner. The solubility of spegazzinine in dilute alkali indicated a free phenolic group and this was confirmed by methylation with diazomethane which led to *O*-methylspegazzinine (III), whose ultraviolet absorption spectrum was identical with that of aspidospermine. The substance (III) could be acetylated and the resulting *O*-methyl-*O*-acetylspegazzinine (VI) exhibited carbonyl bands typical of an amide and an alcoholic acetate. The same product (VI) could also be prepared by methylating deacetylspegazzinine (II) to *O*-methyldeacetylspegazzinine (V) followed by *O,N*-diacetylation. A particularly useful derivative was the nicely crystalline *O*-methyl-*O*-benzoylspegazzinine (VII). Direct acetylation of spegazzinine (I) furnished *O,O*-diacetylspegazzinine (IV) and its infrared spectrum (Fig. 2) shows the presence of phenolic and alcoholic acetates as well as that of an amide.

The presence of a dihydroindole moiety in spegazzinine (I) and its transformation products (II–VI) is clearly demonstrated by the general similarity of the ultraviolet absorption spectra with those of appropriate models[13,14] and other dihydroindole alkaloids.[14,15,16] Spegazzinine is not affected by catalytic hydrogenation and unless a non-reducible double bond is present, three additional rings must be present over and above those shown in partial structure I. It is apparent that no additional benzene ring can be present and on the basis of the spectroscopic evidence at hand and of certain type reactions, it has been possible to locate the phenolic hydroxyl group with a considerable degree of certainty. The evidence for placing it *ortho* to the amino function as in I is as follows:

O-Methyldeacetylspegazzinine (V) gives a magenta color with a solution of ferric chloride in dilute hydrochloric acid, a red color with sodium nitrite in dilute hydrochloric acid (the color turning to yellow on making alkaline) and couples with diazotized sulfanilic acid to give a purple-red color and a scarlet precipitate of a methyl orange on basification. This behavior is similar to that reported for the alkaloids deacetyldiabolin, vomicine,[16] and deacetylaspidospermine,[13] in which a phenolic hydroxyl group in *ortho* position to the dihydroindole amino function is postulated. More importantly, Millson and Robinson[17] have recently carried out a study of the color reactions and ultraviolet absorption spectra of 5-, 6-, 7-, and 8-methoxy-9,11-dimethylhexahydrocarbazole. The color reactions described above and the ultraviolet absorption spectrum of *O*-methyldeacetylspegazzinine (V) exactly parallel the corresponding reactions and spectrum of 8-methoxy-9,11-dimethyl-1,2,3,4,10,11-hexahydrocarbazole but are significantly different from those of the other isomers.

In *O,O*-diacetylspegazzinine (IV) and *O*-methyl-*O*-acetylspegazzinine (VI) the infrared carbonyl band of the amide is at 5.98 μ whereas in spegazzinine the corresponding band occurs near 6.14 μ. Two explanations can be offered, both of them supporting the partial structure I for spegazzinine. The shift is due to hydrogen bonding[6] between the phenolic hydroxyl group and the N-acetyl function in spegazzinine (I) and the absence of such bonding in IV and VI. Alternatively, the interaction resulting from the proximity of the phenolic acetyl and the N-acetyl groups in *O,O*-diacetyl spegazzinine (IV) (and less likely between the methoxyl and N-acetyl groups in VI) may cause a shift of both the phenolic acetyl and N-acetyl carbonyl stretching bands from the normal frequencies; this supposition is supported by the somewhat low wave length for the phenolic acetate band (5.65 μ). *O*-Methylspegazzinine (III) appears to be anomalous in showing an amide carbonyl band at *ca.* 6.12 μ (as does aspidospermine), but no definite conclusion should be based on this point since in this instance the band is rather broad and complex.

Evidence for strong hydrogen bonding between the carbonyl group of the amide and the phenolic hydroxyl in spegazzinine (I) is afforded by the observation that methylation of I with diazomethane in methanol-ether solution is only about 45% complete after six days at 0°, whereas similar methylation of deacetylspegazzinine (II) in which no hydrogen bonding can occur, proceeds to the extent of 95%. The infrared spectrum (Fig. 2) of deacetylspegazzinine (II)[18] shows only a single band in the 3.0 μ region which is probably due to the alcoholic hydroxyl group and the absence of bands in that region corresponding to NH and phenolic OH groups is probably due to internal salt formation between these two substituents. It is appropriate to point out that the infrared spectrum (Fig. 2) of spegazzinine (I) itself in that region shows two bands, in contrast to vomicine and N-acetylaspidosine where none are observed.[6]

In Table I are summarized the pertinent spectroscopic (ultraviolet and infrared) data for spegazzinine (I) and derivatives as well as their specific rotations. Attention should be called to some of the remarkable rotatory changes accompanying appar-

(13) Openshaw and Smith, *Experientia*, **4**, 428 (1948).
(14) Karrer and Schmid, *Angew. Chem.*, **67**, 361 (1955).
(15) Anet and Robinson, *J. Chem. Soc.*, 2253 (1955).
(16) Bader, Schlittler, and Schwarz, *Helv. Chim. Acta*, **36**, 1256 (1953).
(17) Millson and Robinson, *J. Chem. Soc.*, 3362 (1955).

(18) The weak band at 6.14 μ is not due to traces of unreacted amide but rather is part of the aromatic doublet (6.14 and 6.22 μ) which is also found in *O*-methyldeacetylspegazzinine (V), *O,O*-diacetylspegazzinine (IV), and in deacetylaspidospermine (ref. 6).

TABLE I

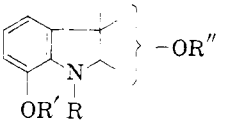
COMPARISON OF ALKALOID SPECTRA

Compound	$[\alpha]_D$($CHCl_3$)	Absorption Spectra Ultraviolet (95% ethanol) λ, mμ	log ε	Infrared ($CHCl_3$)μ
Spegazzinine (I)	+175.6° (*c*, 0.65)	242.5 (min.)	3.82	2.80(w), 2.95(m),
		256.5 (max.)	3.94	6.14(s), 6.26(s),
		285 (infl.)	3.42	6.36(s)
Deacetylspegazzinine (II)	+62.7° (*c*, 0.74)[a]	241 (infl.)	3.87	3.11(m), 6.14(w),
		269 (min.)	3.28	6.22(m).[b]
		290 (max.)	3.48	
O-Methylspegazzinine (III)	−136.1° (*c*, 0.86)	237.5 (min.)	3.74	2.98(m), 6.12(s),
		254 (max.)	3.96	6.23(shoulder, m.)
		280–290 (infl.)	3.42–3.35	
O,O-Diacetylspegazzinine (IV)	+123.1° (*c*, 0.42)	238.5 (min.)	3.93	5.65(s), 5.72(s),
		251.5 (max.)	4.00	5.98(s), 6.08(m),
		281 (infl.)	3.45	6.20(m).
O-Methyldeacetylspegazzinine (V)	−63.5° (*c*, 0.57)	237 (min.)	3.65	2.78(vw), 2.98(m),
		249 (max.)	3.74	6.12(m), 6.21(m).
		278 (min.)	3.29	
		291 (max.)	3.34	
O-Methyl-*O*-acetylspegazzinine (VI)	−22.9° (*c*, 0.55)	237.5 (min.)	3.89	5.73(s), 5.98(s),
		252 (max.)	4.03	6.17(m), 6.21
		275 (infl.)	3.51	(shoulder, m).
		285 (infl.)	3.45	
O-Methyl-*O*-benzoylspegazzinine (VII)	+97.2° (*c*, 0.40)			5.81(s), 6.01(s),
				6.19(m), 6.22(m).
Aspidospermine[c]	−92.8° (*c*, 0.50)	236.5 (min.)	3.71	6.12(s), 6.25(m)
		257 (max.)	4.00	
		280–290 (infl.)	3.48–3.37	

[a] Determined in ethanol-chloroform. (1:4) [b] Measured in mineral oil mull. [c] Our measurements.

ently minor chemical alterations and which are obviously reflections of the interaction of these functional groups.[19] A particularly striking example is the change of 311° associated with methylation of spegazzinine (I → III). Acetylation and particularly benzoylation of the alcoholic hydroxyl group in *O*-methylspegazzinine (III → VI; III → VII) also involves an appreciable rotation change and might indicate a spatial relationship between the alcoholic hydroxyl and the amide function as expressed in VIII.

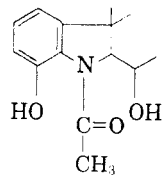
Partial structure I for spegazzinine, the apparent empirical formula $C_{21}H_{28}N_2O_3$ of this alkaloid, and the fact that it is also isolated from the same plant genus as aspidospermine strongly suggest that spegazzinine may be a hydroxylated derivative of demethylaspidospermine.[6] Unfortunately, it has so far been impossible with the amount of spegazzinine at our disposal to prove this relationship by a chemical interconversion. The obvious approach would be to oxidize the alcoholic hydroxyl group in *O*-methylspegazzinine (III) and subsequently eliminate it which should yield aspidospermine or deacetylaspidospermine if the N-acetyl group should be lost during the reduction of the ketone. Direct chromium trioxide oxidation in an acidic medium failed because of precipitation of the amine salt and Oppenauer oxidation of spegazzinine itself yielded unreacted starting material. Oxidation of *O*-methylspegazzinine (III) with chromium trioxide in pyridine led in poor yield to a neutral product (no titratable amino group) but this probably involved oxidation of an adjacent methylene group[20] rather than indicating the presence of a carbinolamine moiety in spegazzinine (I). The rather low pK'_a of 6.0, similar to that of aspidospermine[13] would exclude a carbinolamine unless it is of a type which cannot tautomerize to the quaternary salt; moreover, the alkaloid is not affected by sodium borohydride in boiling methanol solution. Further degradations of spegazzinine are contemplated when additional supplies of the alkaloid become available.



I R = CH_3CO, R′ = R″ = H
II R = R′ = R″ = H
III R = CH_3CO; R′ = CH_3; R″ = H
IV R = R′ = R″ = CH_3CO
V R = R″ = H; R′ = CH_3
VI R = R″ = CH_3CO; R′ = CH_3
VII R = CH_3CO; R′ = CH_3; R″ = C_6H_5CO

(19) Qualitatively, somewhat similar rotation changes also seem to occur in the aspidospermine series. The following rotations (chloroform) were obtained in our laboratory on specimens kindly furnished by Dr. B. Witkop (ref. 6): aspidospermine, $[\alpha]_D$ −93°; demethylaspidospermine perchlorate, $[\alpha]_D$ + 94°; deacetylaspidospermine, $[\alpha]_D$ +24° (ethanol); aspidosine, $[\alpha]_D$ +25°.

(20) See Djerassi, Bowers, and Khastgir, *J. Am. Chem. Soc.*, **78**, 1729 (1956) and T. D. Perrine and L. F. Small, *J. Org. Chem.*, **21**, 111 (1956) for a similar case.



VIII

EXPERIMENTAL[21,22]

Isolation of alkaloids from Aspidosperma chakensis Spegazzini. The air-dried, powdered bark[9] (2 kg.) was extracted four times by refluxing for six hours (with prior standing for 48 hours at room temperature) with 2–3 l. portions of ethanol and the combined extracts were concentrated (first at atmospheric pressure and then *in vacuo*) to a volume of *ca.* 600 cc. The mixture was poured into ice-cold 5% acetic acid and left overnight in the refrigerator in contact with 700 cc. of chloroform. The resinous material was removed by decantation, the remaining solution was made alkaline with ammonium hydroxide, and the bases were taken up in chloroform. The latter solution was again extracted with 5% acetic acid, made basic with ammonia, and re-extracted with chloroform; drying and evaporation left a dark brown alkaloidal fraction which was used for all subsequent work.

This residue was refluxed with 700 cc. of anhydrous ether, leaving 6 g. of insoluble material which was not investigated further. To the cooled ether extract was added an excess of a 10% ethereal solution of anhydrous oxalic acid and the precipitated oxalate was collected and washed with hot ether. The yellowish solid was recrystallized from ethanol to yield 16.2 g. of colorless *spegazzinine oxalate,* m.p. 209–213° (gas evolution).

The reddish alcohol filtrate from the oxalate recrystallization was concentrated, diluted with ammonium hydroxide, and extracted with chloroform. The chloroform layer in turn was shaken repeatedly with 5% acetic acid, made basic with ammonia, and the alkaloid (6.9 g.) was removed with ether. The ether solution was washed with ice-cold 5% potassium hydroxide in order to remove phenols, washed with water, dried, and evaporated; yield, 2.2 g. of brown solid. A 0.5-g. portion was sublimed at 230° and 0.1 mm. whereupon 0.295 g. of crude yellow *quebrachamino* (m.p. 100–122° with sintering at 60°) was obtained.

Characterization of quebrachamine. The crude alkaloid (240 mg.) was recrystallized from ethanol to yield 110 mg. of colorless crystals, m.p. 140–144°, undepressed upon admixture with authentic[11] quebrachamine[10] (m.p. 142–146°), $[\alpha]_{589}$ −110° (dioxane), infrared absorption spectrum identical with that of quebrachamine. The rotatory dispersion curves[23] of the two alkaloid specimens in dioxane solution were practically superimposable over the range 700–320 mμ. Typical values for authentic quebrachamine were as follows: $[\alpha]_{700}$ −71°, $[\alpha]_{650}$ −88°, $[\alpha]_{589}$ −104°, $[\alpha]_{500}$ −150°, $[\alpha]_{475}$ −164°, $[\alpha]_{450}$ −189°, $[\alpha]_{425}$ −221°, $[\alpha]_{400}$ −272°, $[\alpha]_{375}$ −343°, $[\alpha]_{350}$ −448°, $[\alpha]_{337.5}$ −577°, $[\alpha]_{330}$ −712°, $[\alpha]_{325}$ −844°, $[\alpha]_{320}$ −883°. The melting points of the red picrate (m.p. 176–182°) and of the methiodide (m.p. 224–227°) were not depressed when mixed with the corresponding derivatives (m.p. 177–184° and m.p. 227–229°) prepared from authentic quebrachamine and the infrared spectra (nujol) of the two picrate specimens were identical.

Anal. Calc'd for $C_{19}H_{26}N_2$: C, 80.81; H, 9.28; N, 9.92. Found: C, 80.37; H, 9.46; N, 9.74.

Characterization of spegazzinine (I). The analytical sample of spegazzinine *oxalate,* as obtained from the alkaloid fractionation scheme outlined above, was crystallized from methanol-ether and apparently represented the monohydrate; m.p. 212–214° (gas evolution).

Anal. Calc'd for $C_{21}H_{28}N_2O_3.(COOH)_2.H_2O$: C, 59.46; H, 6.94; N, 6.03; Acetyl, 9.27. Found: C, 59.91; H, 7.12; N, 5.95; Acetyl, 8.34; Methoxyl, 0.27; N-methyl, 0.81.[12]

A 1.0-g. sample of the oxalate was suspended in 25 cc. of water, treated with 25 cc. of 4% sodium bicarbonate solution, and extracted with ether. Evaporation of the ethereal solution to dryness and recrystallization from dilute methanol afforded colorless leaflets (0.71 g.) of *spegazzinine* (I), m.p. 104.5–106° (depends on rate of heating and dryness of sample; ranges between 100–112° have been observed), $[\alpha]_D$ +175.6°. The pertinent spectroscopic information is given in Table I and Figs. 1 and 2.

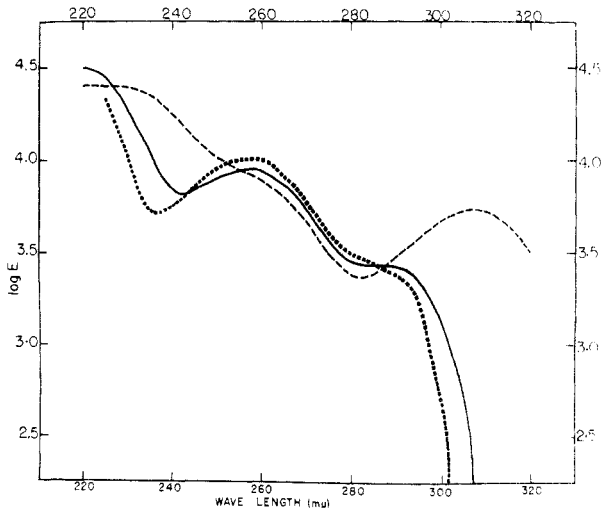


FIG. 1.—ULTRAVIOLET ABSORPTION SPECTRA OF: ———, SPEGAZZININE (I) IN 95% ETHANOL; – – – –, SPEGAZZININE (I) IN 0.2 *N* POTASSIUM HYDROXIDE IN 95% ETHANOL; . . . . . , ASPIDOSPERMINE IN 95% ETHANOL.

Anal. Calc'd for $C_{21}H_{28}N_2O_3$: C, 70.76; H, 7.92; N, 7.86; O, 13.46; Acetyl, 12.06; C-methyl, 4.21; Mol. wt., 340. Found:[24] C, 71.00, 71.01; H, 8.16, 8.21; N, 7.69; O, 14.22; Acetyl, 12.17; C-methyl, 8.67;[25] pK'_a 6.0 and 13.0 ± 0.4[26] in 60% dimethylformamide; apparent mol. wt. (from electrometric titration, 362.

Spegazzinine hydrochloride (1.6 g.) was obtained when 1.75 g. of the alkaloid was dissolved in absolute ethanol and treated with ethanolic hydrogen chloride. The analytical sample was recrystallized from methanol but no definite

(21) We are indebted to Dr. A. E. Lippman for carrying out some preliminary experiments.

(22) Melting points were determined on the Kofler block. Unless noted otherwise, rotations and infrared spectra were measured in chloroform and ultraviolet absorption spectra in 95% ethanol solution. We are grateful to Mrs. Dolores Phillips for all spectroscopic determinations. Microanalyses were carried out by Dr. A. Bernhardt (Mülheim, Germany), Mr. Joseph F. Alicino (Metuchen, New Jersey), Mr. G. M. Maciak (Lilly Research Laboratories), and Spang Microanalytical Laboratory (Plymouth, Michigan).

(23) Determined by Mrs. R. Riniker by the procedure of Djerassi, Foltz, and Lippman, *J. Am. Chem. Soc.,* **77,** 4854 (1955).

(24) On a sample dried to constant weight at 78° *in vacuo.* The weight loss (found: H_2O, 4.71, 4.89) corresponds to one mole of water of crystallization (calc'd H_2O, 4.63).

(25) This corresponds to only one C-methyl group since the acetyl function also shows up in the Kuhn-Roth determination. Under the same conditions, aspidospermine showed C-methyl, 8.44.

(26) Carried out in the Eli Lilly Laboratories through the kindness of Dr. N. Neuss.

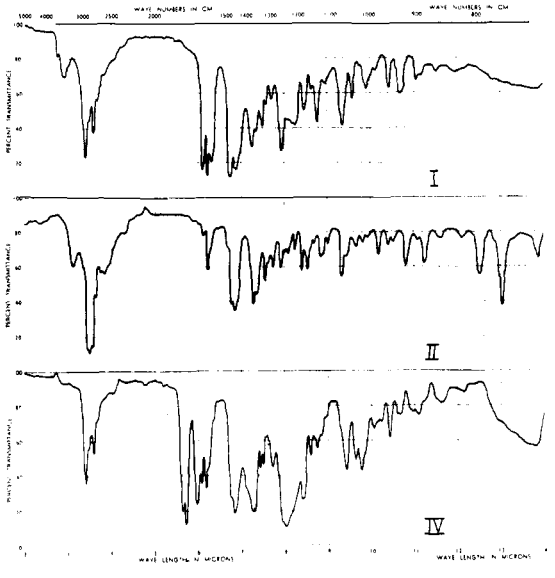


FIG. 2.—INFRARED ABSORPTION SPECTRUM OF: (I) SPEGAZZININE IN CHLOROFORM; DEACETYLSPEGAZZININE (II) IN MINERAL OIL MULL; AND *O,O*-DIACETYLSPEGAZZININE (IV) IN CHLOROFORM.

melting point could be obtained because of decomposition (*ca.* 278–298° starting at 250°).

Anal. Calc'd for $C_{21}H_{29}ClN_2O_3$: C, 64.18; H, 7.43; N, 7.13; Cl, 9.03. Calc'd for $C_{22}H_{31}ClN_2O_3$: C, 64.92; H, 7.67; N, 6.88; Cl, 8.72. Found:[27] C, 64.92; H, 7.63; N, 6.83; Cl, 8.81.

Spegazzinine was recovered unchanged in an attempt to prepare a methiodide by standing at room temperature for 4 days with methyl iodide in methanol solution.

Deacetylspegazzinine (II). A mixture of 200 mg. of spegazzinine, 1.5 cc. of conc'd hydrochloric acid, and 3 cc. of water was refluxed in a current of nitrogen for 3 hours. Ammonium hydroxide was added to the cooled reaction mixture and the resulting precipitate (140 mg., m.p. 250–255°) was collected. This material could be crystallized from dilute ethanol to give rosettes of needles, m.p. 243–257° (with prior sintering and darkening) but since the substance is quite sensitive to atmospheric oxidation, some decomposition (with darkening of the solution) usually occurred during the recrystallization process. Purification was best achieved by sublimation of the crude reaction product at 205° and 0.005 mm. to give a white, micro-crystalline solid, m.p. 254–257° (with decomposition on a stage preheated to 235°), $[\alpha]_D$ +62.7° (chloroform containing 20% of ethanol); for spectroscopic data, see Table I and Fig. 2.

Anal. Calc'd for $C_{19}H_{26}N_2O_2$: C, 72.58; H, 8.34; N, 8.91; O, 10.17; C-methyl, 4.77; Active hydrogen, 0.32 (for one H). Found: C, 72.76; H, 8.62; N, 8.75; O, 11.02; C-methyl, 3.63; Active hydrogen, 0.94.

O-Methylspegazzinine (III). Spegazzinine (I) (0.5 g.), dissolved in 30 cc. of methanol, was treated with 150 cc. of a freshly distilled ethereal solution of diazomethane (prepared from 5.0 g. of N-nitrosomethyl urea) and left at 0° for six days. Excess diazomethane was decomposed by the addition of a few drops of acetic acid and the ether solution then was extracted with 5% sodium hydroxide solution. Neutralization of the alkali extracts yielded 230 mg. of unreacted spegazzinine (I) while from the ether solution, there was obtained 205 mg. of the desired methyl ether III, which could not be crystallized but which distilled unchanged at 167° and 0.03 mm., $[\alpha]_D$ −136°; the spectral characteristics are given in Table I.

Anal. Calc'd for $C_{22}H_{30}N_2O_3$: C, 71.32; H, 8.16; N, 7.56; Basic N, 3.78; O, 12.96; C-methyl, 4.06; O-methyl, 8.38. Found: C, 70.98; H, 7.89; N, 7.69; Basic N (by perchloric acid titration), 4.19; O, 13.19; C-methyl, 5.91;[25] O-methyl, 9.51; N-methyl, 1.04.[12,28]

Spegazzinine could also be methylated with dimethyl sulfate in boiling acetone in the presence of potassium carbonate but the above diazomethane procedure gives a cleaner product.

O,O-Diacetylspegazzinine (IV). Spegazzinine (100 mg.) was refluxed with 2 cc. each of benzene and acetic anhydride for 6 hours and most of the solvent was removed *in vacuo*. The excess acetic anhydride was hydrolyzed by addition of water, the mixture was made alkaline with ammonium hydroxide, and the product was extracted with ether. The resulting acetate could not be crystallized nor could a crystalline picrate or oxalate be prepared and it was consequently distilled at 180° and 0.005 mm., $[\alpha]_D$ +123°. Infrared examination (*cf.* Fig. 2 and Table I) before and after distillation demonstrated that no decomposition had occurred.

Anal. Calc'd for $C_{25}H_{32}N_2O_5$: C, 68.16; H, 7.32; N, 6.36; Basic N, 3.18; 2 O-acetyl, 19.52; Neutral equivalent, 440. Found: C, 68.12; H, 7.06; N, 6.72; Basic N (perchloric acid titration), 3.29; O-acetyl, 20.94 (basic hydrolysis); Neutral equivalent (perchloric acid titration), 425.

O-Methyldeacetylspegazzinine (V). The methylation of 130 mg. of deacetylspegazzinine (II) in 65 cc. of methanol was carried out at 0° for 6 days with 10 cc. of ethereal diazomethane (distilled) derived from 0.6 g. of N-nitroso methylurea and yielded only 5 mg. of phenolic material and 70 mg. of a neutral fraction. As soon as the diazomethane solution had been added to the almost colorless solution of deacetylspegazzinine, a deep red coloration was produced which persisted throughout the methylation. The crude methyl ether, representing a brown gum, could not be crystallized nor could a crystalline picrate or oxalate be prepared, but it could be distilled readily at 140° and 0.01 mm., $[\alpha]_D$ −63.5°; the color reactions of this substance are mentioned in the discussion section while the spectroscopic data are listed in Table I.

Anal. Calc'd for $C_{20}H_{28}N_2O_2$: C, 73.14; H, 8.59; N (total as well as basic), 8.53; O, 9.74; O-methyl, 9.48; Neut. equiv., 164. Found: C, 73.64; H, 8.73; N (perchloric acid titration), 8.11; N (Dumas), 8.71; O, 10.07; O-methyl, 9.74; N-methyl,[12] 2.80; Neut. equiv. (perchloric acid titration), 173.

O-Methyl-O-acetylspegazzinine (VI). (*a*) *By acetylation of O-methylspegazzinine* (III). *O*-Methylspegazzinine (III) (100 mg.) was acetylated with acetic anhydride-benzene as described above for IV and the resulting yellow gum, which could not be crystallized, was distilled at 167° and 0.01 mm. to yield a colorless glass, $[\alpha]_D$ −22.9° (*cf.* Table I).

Anal. Calc'd for $C_{24}H_{32}N_2O_4$: C, 69.88; H, 7.82; N, 6.79; O-methyl, 7.52. Found: C, 69.33; H, 7.85; N, 6.81; O-methyl, 8.98; N-methyl, 0.50.[12]

(*b*) *By acetylation of O-methyldeacetylspegazzinine* (V). The solution became cherry red when 80 mg. of *O*-methyldeacetylspegazzinine (V) was acetylated with acetic anhydride-benzene. Distillation of the reddish oily product (65 mg.) at 165° and 0.01 mm. furnished a nearly colorless glass, which was shown to be identical by means of its infrared spectrum with *O*-methyl-*O*-acetylspegazzinine (VI) prepared by procedure (*a*).

O-Methyl-O-benzoylspegazzinine (VII). *O*-Methylspegazzinine (227 mg.) in 2.5 cc. of dry benzene was left for 2 days

(27) On a sample dried to constant weight at 100° *in vacuo*. Another analysis on a sample dried in the usual manner (but not checked by constant weight determination) gave C, 64.12; H, 7.36; N, 7.01, in good agreement with the C_{21} formulation.

(28) Under the same conditions, aspidospermine ($C_{22}H_{30}N_2O_2$: Calc'd: O-methyl, 8.75; N-methyl (if present; *cf.* ref. 12), 4.22) gave O-methyl, 8.96, 8.97; N-methyl, 1.83, 0.50.

at room temperature with 0.09 cc. of benzoyl chloride. The benzene was removed *in vacuo* and the residue was treated with a solution of 50 mg. of sodium hydroxide in 5 cc. of water. After standing for 5 minutes, the resultant suspension was extracted with ether, washed with 2 *N* hydrochloric acid, and the aqueous phase was made alkaline with ammonium hydroxide and again extracted with ether. Evaporation of the ether and crystallization from methanol gave 25 mg. of the benzoate VII, m.p. 176–182°; chromatography of the mother liquors furnished an additional 40 mg. of benzoate and 102 mg. of recovered methyl ether III. The analytical sample crystallized from methanol as prisms, m.p. 192–194°, $[\alpha]_D$ +97.2°.

Anal. Calc'd for $C_{29}H_{34}N_2O_4$: C, 73.40; H, 7.23; N, 5.90; Mol. wt, 474. Found: C, 73.43; H, 7.61; N, 5.59; pK'_a 5.8 ± 0.2,[26] apparent mol. wt. (from electrometric titration), 488 ± 40.

Attempted dehydration of *O*-methylspegazzinine by pyrolysis of the benzoate VII proved abortive.

Oxidation of O-methylspegazzinine (III). A mixture of 200 mg. of III, 200 mg. of chromium trioxide, and 4 cc. of pyridine was allowed to stand at room temperature overnight, diluted with 100 cc. of chloroform and filtered. Extraction of the chloroform solution with dilute hydrochloric acid followed by addition of ammonia and extraction with ether led to 40 mg. of recovered methyl ether (III). The chloroform solution of the non-basic products was dried and evaporated to give 56 mg. of a viscous oil, which could not be crystallized and which was distilled at 230° and 0.05 mm. Electrometric titration in 66% dimethylformamide solution[26] showed that this substance had no titratable groups between *p*H 3–14 and the infrared spectrum showed bands at 3.0, 5.93, 6.12, 6.28, and 6.72 μ. Not enough material was available for analysis.

An alternative oxidation involved the Oppenauer oxidation (6 hours refluxing) of 100 mg. of spegazzinine (I) with 100 mg. of aluminum isopropoxide, 5 cc. of dioxane, and 1 cc. of cyclohexanone, but 83 mg. of unreacted starting material was recovered.

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