[JOINT CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY AND THE RESEARCH LABORATORIES OF CHAS. PFIZER AND CO.]

Alkaloid Studies. XXII.[1] The Alkaloids of *Vallesia dichotoma* Ruiz et Pav[2]

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In an examination of the alkaloids of *Vallesia dichotoma* there was isolated reserpine, aspidospermine, vallesin, and a new alkaloid ($C_{21-22}H_{24-26}N_2O_4$) now named dichotamine. Analytical data and spectral evidence suggest that the latter compound contains the same methoxylated *N*-acetyldihydroindole nucleus (I) as is present in aspidospermine.

In a recent publication[5] we described the isolation of a new alkaloid spegazzinine ($C_{21-22}H_{28-30}N_2O_3$) which occurs together with quebrachamine in the bark of *Aspidosperma chakensis* Spegazzini. In continuation of our studies into the alkaloids from the *Apocynaceae* plant family we have now undertaken a detailed examination of *Vallesia dichotoma* from which an alkaloid had already been isolated[6] and subsequently shown to be aspidospermine.[7]

In the present investigation all parts of the plant were separately examined by a scheme[8] used earlier with certain *Rauwolfia* alkaloids with the following results:

Reserpine. This alkaloid was isolated in approximately 0.025% yield from the benzene-soluble acetates derived from the plant stems. This represents one of the few recorded instances where this important alkaloid has been encountered in a genus other than *Rauwolfia* and it appears to be the first time that reserpine has been isolated from the genus *Vallesia.* Thus while small amounts of reserpine have been reported in one species each of the genera *Alstonia,*[9a] *Tonduzia*[9b] and *Vinca,*[9c] none has been found in *Vallesia glabra.*[10]

Aspidospermine, isolated in approximately 0.2% yield from the benzene-insoluble acetate fraction from the leaves and twigs. The identity of this compound was established by mixture melting point determination, infrared and ultraviolet spectra.[11]

Vallesin, isolated in approximately 0.04% yield from the benzene-insoluble acetate fraction from the leaves and twigs. This alkaloid was first isolated from *Vallesia glabra* and shown to be identical with desacetylformylaspidospermine.[12] It should be noted that on the basis of the formula $C_{22}H_{30}N_2O_2$ for aspidospermine,[11] the formula for vallesin should be $C_{21}H_{28}N_2O_2$ rather than $C_{20}H_{26}N_2O_2$ as reported[12] originally and our own analytical results are consistent with this view.

Dichotamine, a new alkaloid isolated in approximately 0.05% yield from the benzene-insoluble acetate fraction of the leaves and twigs. The compound has the molecular formula $C_{21-22}H_{24-26}N_2O_4$ and contains one methoxyl, one *N*-methyl (*vide infra*) and one (N- or O-) acetyl group. The ultraviolet absorption spectrum [λ_{min} 235 mμ (log ϵ 3.73), λ_{max} 255 mμ (log ϵ 4.04), λ_{infl} 280–290 mμ (log ϵ 3.43–3.29)] is almost identical with the corresponding spectrum of aspidospermine. The chromophoric system of the latter compound has been shown[13] to be that of the methoxylated *N*-acetyldihydroindole system (I) and it therefore seems likely that dichotamine also contains this chromophore thus accounting for the methoxyl and acetyl groups found in the analysis. The infrared spectrum of dichotamine shows strong absorption bands at 5.67, 6.00, and 6.67 μ and a moderate intensity band at 6.22 μ. There is no significant absorption in the 3 μ region or at 8 μ. The 6.00 μ band is attributable to the *N*-acetyl group of partial structure (I) and the 6.22 μ and 6.67 μ bands are probably due to the aromatic

(1) Paper XXI, C. Djerassi, C. Bankiewicz, A. L. Kapoor, and B. Riniker, *Tetrahedron,* **2**, 168a (1958).

(2) The work at Wayne State University was supported by research grants from Chas. Pfizer and Co. and from the National Heart Institute (grant No. H-2574) of the National Institutes of Health.

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(5) O. O. Orazi, R. A. Corral, J. S. E. Holker and C. Djerassi, *J. Org. Chem.*, **21**, 979 (1956).

(6) V. Carcamo, *Bol. Soc. Quim. Peru,* **2**, 25 (1936); *Chem. Abstr.*, **30**, 6510 (1936).

(7) V. Deulofeu, J. De Langhe, R. Labriola and V. Carcamo, *J. Chem. Soc.*, 1051 (1940).

(8) F. A. Hochstein, K. Murai and W. H. Boegemann, *J. Am. Chem. Soc.*, **77**, 3551 (1955); S. C. Pakrashi, C. Djerassi, R. Wasicky and N. Neuss, *J. Am. Chem. Soc.*, **77**, 6687 (1955).

(9a) W. D. Crow and Y. M. Greet, *Austral. J. Chem.*, **8**, 461 (1955); R. G. Curtis, G. J. Handley and T. C. Somers, *Chem. & Ind. (London)*, 1598 (1955). (b) A. F. St. André, B. Korzun and F. Weinfeldt, *J. Org. Chem.*, **21**, 480 (1956); (c) N. K. Basu and B. Sarkar, *Nature,* **181**, 552 (1958).

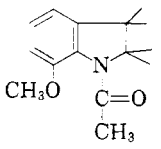
(10) W. J. McAteer, R. G. Weston and E. E. Howe, *Chem. & Ind. (London)*, 1387 (1956). According to some botanical authorities *V. glabra* and *V. dichotoma* are synonyms, but they may represent different varieties of the same species.

(11) B. Witkop, *J. Am. Chem. Soc.*, **70**, 3712 (1948) and earlier references. We are greatly indebted to Dr. B. Witkop (National Institutes of Health, Bethesda) for an authentic specimen.

(12) E. Schlittler and M. Rottenberg, *Helv. Chim. Acta,* **31**, 446 (1948).

(13) B. Witkop and J. B. Patrick, *J. Am. Chem. Soc.*, **76**, 5603 (1954); J. R. Chalmers, H. T. Openshaw, and G. F. Smith, *J. Chem. Soc.*, 1115 (1957).

ring present in (I). In this connection it is significant that aspidospermine shows similar infrared bands in this region of the spectrum. It is interesting to speculate that the band at 5.67 μ could be attributed to a lactone ring and further that the difference in structure between dichotamine and aspidospermine might lie largely in such a lactone. The apparent molecular formulas of aspidospermine ($C_{22}H_{30}N_2O_2$) and dichotamine ($C_{22}H_{26}N_2O_4$) could be consistent with such an hypothesis. Furthermore, we have already presented evidence[5] to show that spegazzinine, which is possibly structurally related to aspidospermine, has an alcoholic hydroxyl group. A similar group in dichotamine could be involved in lactone formation. Functional group analysis points towards the presence of an *N*-methyl group although it is conceivable that this may prove to be erroneous.[14] It is pertinent to note that aspidospermine was originally believed to lack such a functional group, then was assigned one by nuclear magnetic resonance studies,[15] and finally was shown not to possess this moiety by classical degradation means.[16] Additional work is necessary to settle this point and to gain further insight into the structural features of this interesting member of the *Aspidosperma* alkaloid group. Lack of material has so far prevented further degradative study of dichotamine and since no additional supplies are anticipated in the foreseeable future, the results to date are presented in this paper.



I

The many other fractions we have obtained from *V. dichotoma* have so far yielded no crystalline material in characterizable amounts although paper chromatography indicates the presence of other alkaloids.

EXPERIMENTAL[17]

Isolation of alkaloids. Vallesia dichotoma was collected in the Department of Ica, 350 kilometers south of Lima, Peru, by Dr. Ramon Ferreyra (Universidad Nacional Mayor de San Marcos, Lima) to whom we express our thanks. The material consisted of 655 g. of stems, 1300 g. of leaves and twigs, and root which was divided into two portions: 1280 g. of root and 840 g. of low stem material, cut from the "pure" root pieces.

Leaves and twigs (1300 g.) were ground and extracted with 6 l. of boiling methanol for 6 hr., filtered, washed, and the extraction repeated. The combined extracts containing 300 g. solids were concentrated to 1 l. and 300 ml. of glacial acetic acid added. The solid residue was separated by filtration, washed with hexane and with methanol to leave 10.8 g. of a white partially crystalline solid. This non-basic material was not investigated further.

The filtrate was diluted with 4 l. of water and extracted with 4 × 1 l. hexane. The combined extracts were washed with 10% acetic acid and concentrated to dryness to give 26 g. of chlorophyll and waxes which were not further examined.

The acid aqueous phase was then extracted with 4 × 1 l. benzene, the extract washed with 10% ammonium hydroxide and concentrated to dryness under vacuum to give 2.0 g. solids. An acetic acid solution gave a positive Meyer's test. Paper chromatography showed no reserpine and chromatography on alumina gave no crystalline material.

The acid aqueous phase from above was then basified to *p*H 10.7 and extracted with 3 × 1500 ml. chloroform. Concentration to dryness under vacuum yielded 12.5 g. solid. Paper chromatography showed the presence of at least 6 components. This solid (7.25 g.) was dissolved in 500 ml. chloroform:benzene (1:1) and, after removal of a small amount of amorphous material, the solution was chromatographed on 800 g. alumina, collecting fractions of 200 ml. The first 6 fractions eluted with benzene:chloroform (1:1) contained only 69 mg. of material and were discarded.

From fractions 7–14 [benzene:chloroform (1:1)], 2.1 g. of brown, partially crystalline material was obtained. Crystallization from a small amount of acetonitrile gave 470 mg. of colorless prisms, m.p. 180–195°. Twice recrystallized from ethyl acetate and once from aqueous methanol the above material had m.p. 195–200°, $[\alpha]_D$ −92.5° (*c*, 0.58%), λ_{max}, 256 mμ (log ϵ 4.01), λ_{min} 236 mμ (log ϵ 3.68), λ_{infl} 280–290 mμ (log ϵ 3.40–3.18). These constants and the infrared spectrum of the compound are identical with those of *aspidospermine* and a mixture melting point showed no depression.

From fractions 14–21 [benzene:chloroform (1:1)], 21–28 [benzene:chloroform (1:3)], and 29 (chloroform) intractable oils, (410 mg.), (395 mg.), and (160 mg.) respectively were isolated. Fractions 30–46 (chloroform) gave 672 mg. of a yellow oil which solidified on trituration with a small amount of methanol and which was crystallized from acetonitrile to give *dichotamine* in needles, m.p. 225–249°. After many recrystallizations from ethyl acetate and from aqueous methanol dichotamine had m.p. 254–257°; $[\alpha]_D$ −105.2° (*c*, 0.74%); pK_a 5.4 and 10.8 in 66% dimethylformamide, mol. wt. 340; λ_{max} 255 mμ (log ϵ 4.04), λ_{min} 235 mμ (log ϵ 3.75), λ_{infl} 280–290 mμ (log ϵ 3.43–3.29); infrared bands (chloroform solution) *inter al.* at 5.67 (s), 6.00 (s), 6.22 (m) and 6.67 (s) μ.

Anal. Calcd. for $C_{22}H_{26}N_2O_4$: C, 69.09; H, 6.85; N, 7.37; OMe, 8.1; (N)-Me, 3.9; CH_3CO, 11.2; mol. wt. 382.4; for $C_{21}H_{24}N_2O_4$: C, 68.46; H, 6.57; N, 7.60; OMe, 8.42; (N)-Me, 4.08; CH_3CO, 11.69; mol. wt. 368.4. Found: C, 68.47; H, 6.68; N, 7.51; OMe, 7.15; (N)-Me, 3.22; CH_3CO, 9.11.

From fractions 22–29 [chloroform:methanol (99:1)], 30–36 [chloroform:methanol (9:1)], and 37–39 (methanol) 1.32 g. of intractable material was obtained.

From a second batch of leaves and twigs (1300 g.) 19 g. of chloroform-soluble bases were obtained by the above extraction procedure. As it was felt that some of the less polar constituents of the mixture may not have been purified sufficiently for characterization in the above chromatography on partially deactivated alumina, the material from the

(14) See B. Witkop, *J. Am. Chem. Soc.*, **71**, 2559 (1949).

(15) H. Conroy, P. R. Brook, M. K. Rout, and N. Silverman, *J. Am. Chem. Soc.*, **79**, 1763 (1957).

(16) H. Conroy, P. R. Brook, M. K. Rout; and N. Silverman, *J. Am. Chem. Soc.*, **80**, 5178 (1958). We are indebted to Dr. Conroy for an advance copy of his manuscript.

(17) All melting points were determined with a Kofler block. Rotations and infrared spectra were measured in chloroform and ultraviolet spectra in 95% ethanol. Unless otherwise stated, alumina used in chromatography was of chromatographic grade and was partially deactivated by shaking a benzene slurry of 100 g. of alumina with 3 ml. of 10% aqueous acetic acid. We are grateful to Mrs. D. Phillips for all spectroscopic determinations and to Mr. G. M. Maciak (Lilly Research Laboratories) and to Dr. A. Bernhardt (Mulheim, Germany) for microanalytical determinations.

second extraction (7.25 g.) was chromatographed on 800 g. of undeactivated Merck acid washed alumina, collecting fractions of 500 ml. Fractions 1–22 [benzene:chloroform (1:1)] and 23–25 [benzene:chloroform (1:2)] and 25–34 [benzene:chloroform (1:3)] gave 0.27 g., 0.03 g., and 0.34 g. respectively of intractable material.

Fractions 35–46 (chloroform) gave 1.06 g. of a crystalline brown solid. Recrystallized from ether 0.32 g. of *vallesin* was obtained in colorless needles, m.p. 153–156° not raised by further crystallization from hexane or by sublimation; $[\alpha]_D$ −92°, (*c*, 0.12 in chloroform); λ_{max} 259 mμ (log ε 4.11), infrared bands (chloroform) *inter al.* at 5.96 (vs), 6.02 (vs), 6.13 (s), 6.2 (vs), 6.66 (vs) and 6.76 (vs) μ.

Anal. Calcd. for $C_{21}H_{28}N_2O_2$: C, 74.08; H, 8.29; N, 8.23; O, 9.40; OMe, 9.11; (N)-Me, 4.42. Found: C, 73.97; H, 8.18; N, 8.21; O, 9.55; OMe, 8.72; (N)-Me, 1.49.

Fractions 47–51 [chloroform:methanol (97:3)] gave 2.14 g. of a brown semi-solid compound which upon trituration with ether gave 1.18 g. of a cream-colored crystalline compound. Recrystallized from hexane:benzene this material yielded aspidospermine, m.p. 206–208°, characterized by mixture melting point, infrared and ultraviolet spectra.

Fractions 51–62 [chloroform:methanol (95:5)], 63–67 [chloroform:methanol (9:1)], 68–75 [chloroform:methanol (4:1)], 76–82 [chloroform:methanol (7:3)] and 83–96 [chloroform:methanol (2:3)] provided respectively 0.67 g., 0.47 g., 0.21 g., 0.20 g., and 0.37 g. of brown amorphous solid from which no crystalline material could be obtained. It therefore seems likely that dichotamine present in the original mixture is too strongly adsorbed on undeactivated alumina to permit a satisfactory isolation and purification.

Stems. Using an extraction procedure exactly similar to that described for the leaves and twigs, 655 g. of ground stems gave 67 g. of total solid extract, which was processed as described above to give the following fractions: (1) A non-basic extract of hexane solubles (discarded). (2) Benzene-soluble acetates (1.6 g.). (3) Chloroform-soluble bases (2.2 g.). The paper chromatographic behavior of this fraction showed that it had a similar composition to the corresponding fraction from the leaves and twigs.

The benzene-soluble acetates were crystallized from 40 ml. of methanol giving 315 mg. of crude *reserpine*. After washing with hexane, the crude reserpine was recrystallized from methanol:benzene to give 115 mg. of still impure reserpine, m.p. 253–255° (dec.). The infrared spectrum and paper chromatogram were characteristic of authentic reserpine.

The benzene-soluble acetates were chromatographed on alumina but no crystalline fractions were obtained.

Stems taken from directly above the roots, (837 g.) were ground, extracted with methanol and processed as above to give the following fractions: (1) Non-basic hexane extract (5.7 g.) (not investigated). (2) Benzene-soluble acetates (0.80 g.). (3) Chloroform-soluble bases (5.5 g.). (4) Chloroform and water-insoluble bases (4.6 g.) (not investigated).

The benzene-soluble acetates showed no reserpine by paper chromatography.

The chloroform-soluble bases showed 6 components by paper chromatography but none of them appeared to be identical with those of the benzene-insoluble acetates from the previous stem extraction. Careful chromatography of this fraction on alumina, followed by counter-current partition of some of the chromatographic fractions gave only traces of crystalline material in amounts insufficient to characterize.

Roots (1220 g.) were ground, extracted with 2 × 6l. of hot methanol, and the extract (150 g.) was processed as previously described to give the following fractions: (1) Non-basic extract of hexane-solubles (9.2 g.) (not investigated). (2) Benzene-soluble acetates (1.05 g.). (3) Chloroform-soluble bases (8.55 g.).

The benzene-soluble acetates when chromatographed on paper showed 9 components but only traces of reserpine.

The chloroform-soluble bases showed 4 or more components when chromatographed on paper, but careful chromatography on alumina gave only intractable resins.

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[CONTRIBUTION FROM THE INDIAN ASSOCIATION FOR THE CULTIVATION OF SCIENCE]

Studies on the Ultraviolet Absorption Spectra of Coumarins and Chromones. II. Hydroxy Derivatives[1]

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Study of the ultraviolet absorption spectra of coumarins and chromones, substituted by hydroxy groups in the aromatic as well as in the heterocyclic nucleus in different positions shows bathochromic shift in the position of one or more of the principal bands.

In a previous communication[2] it has been shown that a methyl group substituting different positions in coumarin and chromone fails to cause any significant bathochromic shift of the main absorption bands. This was considered to be due to the weak auxochromic property of the methyl group.

The subject of the present investigation is a systematic survey of the absorption characteristics of coumarins and chromones having hydroxyl groups at different positions of the coumarin and chromone molecule.

The absorption spectra of some similar coumarins and chromones have been reported.[3–5]

The compounds studied have been listed in Tables I and II. Absorption was measured with a Beckmann Model DU quartz spectrophotometer

(1) Taken from the thesis submitted by Kalyanmay Sen for the degree of Doctor of Philosophy (Science) of the University of Calcutta, April 1957.

(2) B. K. Ganguly and P. Bagchi, *J. Org. Chem.*, **21**, 1415 (1956).

(3) E. Cingolani, A. Schiavello, and C. Sebastini, *Gazz. chim. ital.*, **83**, 647 (1953).

(4) E. Cingolani, *Gazz. chim. ital.*, **84**, 825 (1954).

(5) E. Cingolani and A. Gaudiano, *Rend. ist. super sanità*, **19**, 1256 (1956).