ON THE ALKALOIDS OF *STRYCHNOS*. XXXV. THE OCCURRENCE OF AKAGERINE IN SOUTH AMERICAN *STRYCHNOS*[1]

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ABSTRACT.—Akagerine (**3**) was isolated from three different species of South American *Strychnos:* from *S. gardneri* A. D.C. (Rio de Janeiro, Brazil), together with 11-methoxydiaboline (**2**); from *S. jobertiana* Baillon (Brazil), together with diaboline (**1**); and from *S. parvifolia* D.C. (Bahia, Brazil).

In recent years a large number of new indole alkaloids have been isolated from Loganiaceae, mainly from the *Strychnos* genus (15). Biogenetic relationships between the various alkaloids of this family and the structurally related alkaloids of the Apocynaceae were suggested and partially confirmed (16).

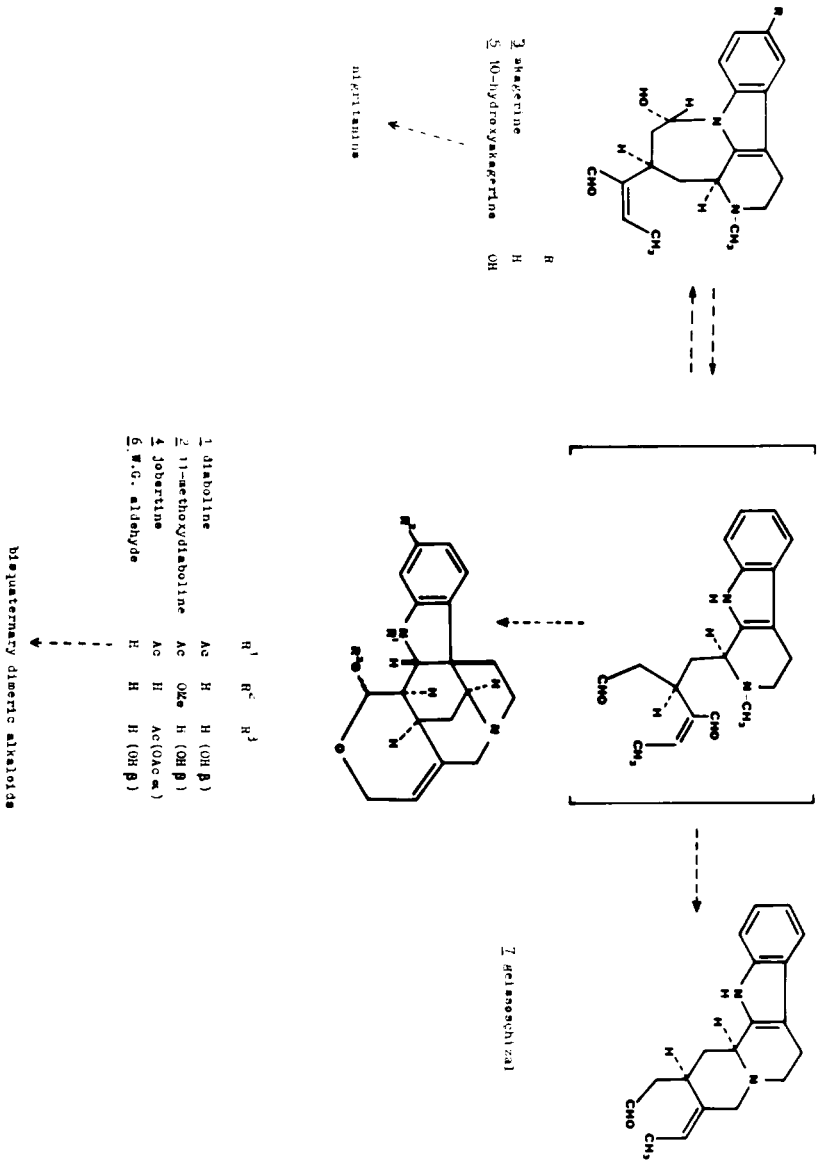
In the course of our research, diaboline (**1**), C-curarine, and other quaternary dimeric alkaloids were isolated from the root bark of a sample of *Strychnos gardneri* A. D.C. (Prance et Silva 58252), collected in the State of Goias in Central Brazil (17). A sample of root bark of the same species, collected in the State of Rio de Janeiro (Grajaù) was reinvestigated. We identified two tertiary alkaloids, 11-methoxydiaboline (**2**), previously found in many American *Strychnos* species (13), and, surprisingly, akagerine (**3**), so far isolated from African *Strychnos* only.

From the root bark of another South American *Strychnos*, *S. parvifolia* D.C., collected in the State of Bahia (Brazil), akagerine (**3**) was isolated as the sole tertiary alkaloid present, whereas several quaternary alkaloids, still under examination, were detected by tlc and paper chromatographic analysis. A previous sample of *S. parvifolia* D.C., erroneously identified as *rubiginosa* (15), collected by Ducke in the State of Pernambuco (Brazil), showed a good curarizing activity. Several known quaternary alkaloids were detected, such as calebassine, alkaloid I, fluorocurarine, fluorocurinine, and mavacurine (18). Tertiary alkaloids of this sample have not been examined.

Finally, another South American *Strychnos*, *S. jobertiana* Baillon, was examined. From the root bark of this sample, collected in the State of Bahia (Brazil), diaboline (**1**) and akagerine (**3**) were isolated, together with quaternary alkaloids still under examination. In previous works on *S. jobertiana*, the presence of quaternary alkaloids having a certain curarizing activity were found in the first sample, collected by Ducke near Manaos (Brazil) (19); whereas, in a second sample, only two tertiary alkaloids, diaboline (**1**) and jobertine (O-acetyldiaboline B) (**4**), were identified (20).

So far, akagerine (**3**) has been found in *S. usambarensis* by Angenot *et al.* (21), in *S. camptoneura* by Verpoorte *et al.* (22), and, together with the isomer kribine,

[1]For the papers I–XIX see ref. 1. Subsequently the following *Strychnos* species have been investigated: *S. nux vomica* L. (2, 7, 11), *S. panamensis* Seem. (3), *S. romeu-belenii* Krukoff and Barneby (4), *S. tabascana* Sprague and Sandwith (5), *S. medeola* Sagot ex Progel (6), *S. amazonica* Krukoff (8), *S. brachiata* Ruitz et Pavon (8), *S. fendleri* Sprague and Sandwith (9, 12), *S. nigritana* Bak (10), *S. rubiginosa* D.C. (13), *S. spinosa* Lam (14).



in *S. dale* and *S. elaeocarpa* by Rolfsen *et al.* (23). We have isolated akagerine, kribine, and 10-hydroxyakagerine (**5**) from *S. spinosa* (14) and again akagerine, kribine and dimeric tertiary alkaloids, the nigritanins, from *S. nigritana* (10). These results enabled us to propose that akagerine could be the intermediary of the biosynthesis of nigritanins (24).

The simultaneous occurrence of akagerine in American and African *Strychnos*, together with strychnine-type of alkaloids (diaboline, curarine), suggests its possible role in the biogenetic pathway of these alkaloids. In fact, akagerine may be correlated with W.G. aldehyde (**6**), the immediate precursor of diaboline and

bisquaternary alkaloids; however, so far the only biogenetic pathway for *Strychnos* alkaloids, demonstrated by feeding experiments, has geissoschizine as the key intermediary (25).

The correlation is easier between geissoschizal (**7**) (and other secondary metabolites of Loganiaceae and Apocynaceae as normacusine B and alstonidine) and akagerine through the opening of the azacyclopentane ring and loss of the Me-N group (see figure).

EXPERIMENTAL[2]

MATERIAL.—*S. gardneri* A. D.C., root bark collected in the State of Rio de Janeiro (Grajaù) (Brazil) by L. E. De Mello filho, 200 g; *S. jobertiana* Baillon, root bark collected in Brazil, Prance 3943, 600 g; *S. parvifolia* D.C., root bark collected in the State of Bahia (Brazil), S. A. Mori and T. S. dos Santos 11795, 700 g.

EXTRACTION.—The root bark was powdered and extracted with 2% aqueous acetic acid until a negative Dragendorff reaction occurred. The pooled liquids were made alkaline to pH 8.5 with sodium carbonate. The alkaline solution was extracted with chloroform, and the solvent was then evaporated *in vacuo*. From the aqueous phase, acidified at pH 5, the quaternary alkaloids were precipitated by the addition of potassium mercuric iodide (Valser reagent). The precipitate was separated by filtration on a gooch filter, and washed several times with water. The solid was dissolved in acetone-H_2O (1:1, v/v); the solution was percolated through a column of Amberlite IRA 400 (Cl^-) to convert the compounds into the corresponding chlorides.

SEPARATION OF TERTIARY ALKALOIDS BY COUNTER CURRENT DISTRIBUTION (CCD).—The chloroform extract was separated by CCD between chloroform and a phosphate buffer (mobile phase) at the reported pH (see below) (26) in a Craig Post apparatus (200 stages, 10:10 ml, upper and lower phase). The separation was followed by tlc on silica gel F_{254} with the solvent benzene-ethyl acetate-diethylamine (5:4:1, v/v/v). Alkaloids were extracted with chloroform from the aqueous phase after alkalinization with the sodium carbonate. The identification was made by the analysis of their spectroscopic data (uv, ir, nmr, ms and ord) and by direct comparison with authentic specimens.

RESULTS

S. gardneri: 11-methoxydiaboline (**2**), purified at pH 6.4 ($K_rK_b = 6 \times 10^{-8}$), 90 mg, mp 214–6° C (lit. 216° (4)) from ethyl acetate, $C_{22}H_{26}N_2O_4$, M^+ at m/z 382, $[\alpha]^{20}D = +20$ (c 0.5, $CHCl_3$); akagerine (**3**), purified at pH 5.2 ($K_rK_b = 7 \times 10^{-1}$)[8], 150 mg, mp 185–6° C (lit. 188° (21)) from ethyl acetate-hexane, $C_{20}H_{24}N_2O_2$, M^+ an m/z 324, $[\alpha^{20}]D = -11$ (c 0.5, $CHCl_3$); quaternary alkaloids (Cl^-): 1 g.

S. jobertiana: diaboline (**1**), purified at pH 6.4 ($K_rK_b = 9 \times 10^{-8}$), 520 mg, mp 187–9° C (lit. 189° (27)) from ethyl acetate, $C_{21}H_{24}N_2O_3$, M^+ at m/z 352, $[\alpha]^{20}D = +36$ (c 0.8, $CHCl_3$); akagerine, 2850 mg; quaternary alkaloids (Cl^-): 1.5 g.

S. parvifolia: akagerine, 4550 mg; quaternary alkaloids (Cl^-): 6 g.

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[2]Mass spectra were recorded with a LKB 9000S spectrometer, nmr spectra with a Varian T60 apparatus (in $CDCl_3$, TMS as internal reference), ord curves with a Cary 60 spectrophotometer.

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