

# Biological and Phytochemical Evaluation of Plants XII: Isolation of Acetylakuammidine from *Aspidosperma quebracho-blanco* Leaves

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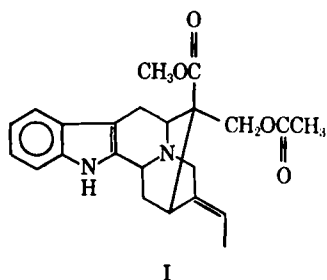
**Abstract** □ A continuing investigation of the leaves of *Aspidosperma quebracho-blanco* Schlecht. (Apocynaceae) has resulted in the isolation of natural acetylakuammidine, in addition to the previously reported alkaloids aspidospermine and quebrachidine. Methods used for the isolation and characterization of these bases are described.

**Keyphrases** □ *Aspidosperma quebracho-blanco* Schlecht. (Apocynaceae) leaves—biological and phytochemical evaluation, isolation of acetylakuammidine □ Acetylakuammidine— isolation from *Aspidosperma quebracho-blanco* leaves □ Phytochemical evaluation—*Aspidosperma quebracho-blanco* leaves, isolation of natural acetylakuammidine

In a previous article, the isolation of aspidospermine, quebrachidine, (–)-pyrifolidine, akuammidine, and rhazinilam from the leaves of *Aspidosperma quebracho-blanco* was reported (1). Rhazinilam, a new type of alkaloid from the Apocynaceae, was subsequently studied and its structure was solved by X-ray crystallography (2). An investigation of the chloroform-soluble Fraction C, currently reported, has yielded acetylakuammidine, as well as additional large quantities of aspidospermine and quebrachidine. Until now, acetylakuammidine (I) has only been prepared synthetically (3).

## EXPERIMENTAL

**Preparation and Chromatography of Alkaloid Fraction**—Two hundred grams of the alkaloid Fraction C (1) was dissolved in ether and partitioned four times with two volumes of 10% acetic acid. The acid solution was separated, evaporated *in vacuo* to remove entrained ether, and placed into five cellulose dialysis tubes, each 100 × 2.65 cm. The tubes, each containing 600 ml. of the acidic alkaloid solution, were suspended in a 9 × 115-cm. glass column, with distilled water being perfused at a rate of 150 ml./hr. at 5°. A total of 9 l. of the dialysate was collected, made alkaline with ammonium hydroxide, and extracted with ether. The ether extract was dried over anhydrous magnesium sulfate, and 40.0 g. of aspidospermine was obtained by direct crystallization of the fraction. It corresponded in all respects with a reference sample of aspidospermine previously isolated from this plant. Removal of the ether from the alkaloid mother liquor yielded 75.0 g. of residue, which was chromatographed over a column of 3.2 kg. of silica gel PF<sub>254</sub> as



previously described (1), using a solvent mixture of benzene–ethyl acetate–95% ethanol (2:2:1).

**Isolation of Acetylakuammidine**—Using methanol as the solvent, 0.327 g. of an alkaloid was obtained as colorless prisms from fractions 48–56 from the column. Concentration of the mother liquor yielded an additional 0.073 g. of the same alkaloid. Recrystallization of the pooled crystals from methanol afforded an analytical sample which sublimed at 245°, and the sublimate subsequently melted at 278–279° dec. The isolate exhibited  $[\alpha]_D^{25} + 13^\circ$  (c 1.0, chloroform), and its UV spectrum in methanol exhibited absorptions at  $\lambda_{max}$  225 (log  $\epsilon$  4.80), 278 (4.06), and 291 (3.96) nm., which were indicative of a tetrahydro- $\beta$ -carboline base. The IR spectrum (KBr) resembled that of akuammidine except for the presence of the acetate absorptions (1735 and 1260  $cm^{-1}$ ). The mass spectrum revealed a molecular ion at  $m/e$  394 ( $C_{23}H_{26}N_2O_4$ ), and the fragmentation pattern of ionic species at  $m/e$  168, 169, 182, 249, 321, and 335 confirmed the presence of the akuammidine nucleus. The base peak at  $m/e$  335 conforms to the  $M - 56$  fragment reported for acetylakuammidine (akuammidine acetate) (3). A mixed melting-point determination with a reference sample of synthetic acetylakuammidine<sup>1</sup> showed no depression, and the IR spectra of the isolate and the reference samples were superimposable, thus confirming the identity of the isolate as acetylakuammidine (I).

**Isolation of Aspidospermine and Quebrachidine**—Fractions 63–72 and 73–100 from the column yielded 1.59 and 2.50 g., respectively, of aspidospermine from methanol, whereas fractions 131–200 afforded 0.81 g. of quebrachidine from the same solvent. Identification of these bases was made by comparison of their physical data with those of reference samples.

## SUMMARY

Acetylakuammidine, quebrachidine, and aspidospermine have been reported isolated from the leaves of *Aspidosperma quebracho-blanco*. This is the first report of the natural occurrence of acetylakuammidine<sup>2</sup> in nature.

## REFERENCES

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<sup>2</sup> Reaction of akuammidine with 10% acetic acid at room temperature did not produce synthetic acetylakuammidine, when analyzed at 24 and 48 hr. and at 7 and 14 days.