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14-KETOALSTONIDINE AND OTHER ALKALOIDAL CONSTITUENTS OF THE STEM BARK OF *ALSTONIA CONSTRICTA*

KARIMAN ALLAM, JOHN A. BEUTLER, and PHILIP W. LE QUESNE

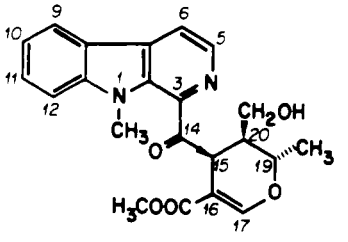
Department of Chemistry, Northeastern University, Boston, Massachusetts 02115

ABSTRACT.—Separation and purification of constituents of the stem bark extract from *Alstonia constricta* yielded eight alkaloids. Some of these alkaloids had been previously isolated from the root bark of this species (alstonilidine, alstonidine, and *O*-3,4,5-trimethoxybenzoylquebrachidine), but others (vincamedine, 1-carbomethoxycarboline, and quebrachidine) had not. A new alkaloid, 14-ketoalstonidine [**1**], is described.

Although the alkaloidal constituents of the root bark of *Alstonia constricta* F. Muell. (Apocynaceae) (1) have been known for many years, the stem bark appears not to have been investigated. We have now isolated eight alkaloids, one of them new, from this source. The separation scheme used was mainly dependent on the different polarity of the alkaloids in the various fractions. The ground bark after removal of lipid materials via hexane extraction was extracted with MeOH. The MeOH extract was extracted with 5% HCl, the aqueous acidic extract was basified with NH_4OH, and the free bases were extracted from the aqueous basic extract first with Et_2O and then $CHCl_3$.

The Et_2O extract was further partitioned between buffer (pH 3) and $CHCl_3$. The aqueous buffer extract was basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract was separated by low pressure column chromatography into six fractions (A-F). Tlc of these fractions showed that neither tetrahydroalstonine nor alstoniline was present.

By gradient elution flash column chromatography (Si gel) of Fraction A and tlc of the subfractions, the known alkaloid, alstonilidine, was obtained, as well as a new alkaloid, identified as 14-ketoalstonidine [**1**].



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14-Ketoalstonidine, a labile, non-crystalline compound, was purified by flash column chromatography and preparative tlc. The uv absorption spectrum in EtOH (λ max 226, 292, 376 nm) suggested the presence of a carbonyl group conjugated to a harman nucleus. (2) The ir spectrum showed the presence of an O-H group (3220 cm^{-1}), and strong bands at 1720, 1695, and 1635 cm^{-1} indicated the presence of two carbonyl groups and an enol ether double bond. The ^{1}H-nmr spectrum of the new alkaloid was similar to that of alstonidine. The aromatic region was identical except that the C6-H signal was shifted downfield. The signals are assigned as follows (the chemical shifts shown in brackets are the corresponding ones in alstonidine). A one-proton doublet at δ 8.45 (8.39) ($J_{5\text{-}6}$=5.0 Hz) is assigned to H-5, a one-proton doublet at δ 8.22 (7.91) ($J_{6\text{-}5}$=5.0 Hz) is assigned to H-6, a one-proton broad doublet at δ 8.18 (8.80) ($J_{9,10}$=7.8 Hz) is assigned to H-9, a one-proton octet at δ 7.67 (7.68) ($J_{10,11}$=8.3, $J_{10,9}$=7.8, $J_{10,12}$=1.3 Hz) is assigned to H-10, a one-proton broad doublet at δ 7.52 (7.48) ($J_{12,11}$=8.4 Hz) to H-12, a one-proton multiplet at δ 7.34 ($J_{11,12}$=8.4,

$J_{11,10}$=8.3, $J_{11,9}$=1 Hz) to H-11, a one-proton singlet at δ 7.87 (7.69) to H-17, and a one-proton signal (d,d,d) at δ 4.63 ($J_{19,20}$=10, $J_{19\text{-}H,Me}$=6.3 Hz) to H-19. The methyl ester signal appeared as a singlet at δ 3.52 (3.44). A one-proton multiplet at δ 2.23 (2.22) is assigned to H-20. The 19-Me group appeared as a doublet at δ 1.40 (1.49) ($J_{19\text{-}Me,H}$=6.4 Hz).

Lamberton (2) reported that the signal from H-15 of alstonidine acetate was obscured by that of the methyl ester at δ 3.68. The new alkaloid gave a signal at δ 5.08 (doublet, $J_{15,20}$=4.9 Hz) which we assigned to H-15. This represents a downfield shift relative to that in alstonidine acetate. The coupling constant, 4.9 Hz, is ascribed to a pseudo-*cis* relationship of H-15 and H-20. The signals at δ 3.80 and 3.36 are assigned to the two C21 hydrogens. The Hb-21 signal appeared as a doublet of doublets (J_{gem}=11.7 and $J_{Hb,H\text{-}20}$=4.2 Hz) at δ 3.80, and the Ha-21 signal appeared as a triplet (doublet of doublets, J_{gem}=$J_{Ha,20\text{-}H}$=11.7 Hz). The C21 hydrogens are not equivalent, owing, we believe, to an intramolecular hydrogen bonding between C21-OH and either the carbonyl group at C14 or Nb of the β-carboline nucleus. Therefore, restricted rotation was expected. From models the angle between H-20 and Ha-21 is ≃170°, that between H-20 and Hb-21 is ≃170°, and that between H-20 and Hb-21 is ≃40°.

A high resolution mass spectrum showed the molecular ion peak at *m/z* 394.15329 ($C_{22}H_{22}N_2O_5$ requires 394.15288). Further support for the assigned structure for this alkaloid was shown by its fragmentation pattern in the mass spectrum as shown in Scheme 1.

Vincamedine was separated from other subfractions of fraction A by column chromatography.

The known alkaloid alstonidine, presumably the biogenetic precursor of the 14-keto-derivative, was crystallized from fraction B. Evaporation of the mother liquor of fraction B followed by flash column chromatography gave 1-carbomethoxy-β-carboline as a major alkaloid. A second alkaloid was separated by prolonged chromatography (flash column chromatography, tlc, and hplc), and identified as *O*-3,4,5-trimethoxybenzoylquebrachidine. Quebrachidine was separated from fraction C.

3,4,5-Trimethoxybenzamide was separated from the first set of fractions from the low pressure column chromatography of the $CHCl_3$-soluble extract. It is likely that this compound is an artifact arising from 3,4,5-trimethoxybenzoylquebrachidine during processing of the alkaloidal extracts with NH_4OH.

EXPERIMENTAL

MATERIALS AND METHODS.—The *A. constricta* stem bark was obtained from Dr. John A. Lamberton, CSIRO, Melbourne, Australia. A voucher specimen is deposited in the herbarium of CSIRO, Melbourne. Standard samples of reserpine, α-yohimbine, tetrahydroalstonine, and alstoniline were obtained from the collections of the late Professor Robert C. Elderfield and Eli Lilly, Inc. Ir spectra were taken on a Perkin-Elmer Model X99 spectrophotometer and absorptions reported in wave numbers (cm^{-1}). The ^1H-nmr spectra were recorded on Varian T-60 and Bruker WM-500 spectrometers, and chemical shift data are reported in parts per million (δ) downfield from TMS used as an internal standard.

Low resolution mass spectra were obtained using a Nuclide 12.19.G instrument. The high resolution mass spectra were obtained on a C.E.C. 21-110B instrument. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. All tlc was performed using precoated, prescored Si plates (GHLF 250μ thick, Analtech). Visualization of chromatograms was effected with uv light (long and short wavelengths), iodine vapor, Dragendorff's reagent, and cerium (IV) ammonium sulfate reagent (1% solution of ceric ammonium sulfate in 85% phosphoric acid). Preparative tlc was done using 20×20 cm, 0.25 mm and 2 mm thick precoated Si plates (E. Merck). Flash column chromatography was performed according to the procedure of Still (3) (Si gel 60, 230-400 mesh, EM Reagents).

EXTRACTION OF THE ALKALOIDS FROM *A. CONSTRICTA* STEM BARK.—The stem bark of *A. constricta* (14 kg) was mixed with heptanes. After filtration the bark was soaked with occasional warming and

mixing in MeOH. The MeOH extract was concentrated to about ¼ by evaporation under reduced pressure.

Separation of the Alkaloids from Non Basic-Compounds.—After moistening with MeOH the dry MeOH extract (510 g) was extracted with 5% HCl solution (5 liters) for 5 h using mechanical stirring. After filtration the acidic aqueous extract was basified with concentrated NH_4OH and extracted with Et_2O. The Et_2O extract was partially concentrated, washed with H_2O, and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave 8.5 g of tertiary base alkaloids. The basified aqueous extract was extracted again with $CHCl_3$ and the organic layer washed with H_2O, then dried (anhydrous Na_2SO_4) and evaporated to dryness, to leave 14 g of Et_2O-insoluble basic material.

Fractionation of the Et_2O Extract.—The Et_2O-soluble alkaloidal extract was evaporated and partitioned between pH-3 phosphate buffer and $CHCl_3$. The $CHCl_3$ solution was evaporated, and the aqueous buffer extract was basified with NH_4OH, re-extracted with $CHCl_3$, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated.

Two 50×2.5 cm columns packed with 200-325 mesh Si gel were prepared with $CHCl_3$-MeOH (9:1). The pH 3-$CHCl_3$-soluble alkaloids (3.01 g) were dissolved in 30 ml of the solvent and chromatographed using these columns. After collection of 80 20-ml fractions, pure MeOH was used to elute the remaining materials. The fractions were designated as follows: Fraction A, Nos. 1-9, 712 mg; Fraction B, Nos. 10-32, 1,474 g; Fraction C, Nos. 33-49, 625 mg; Fraction D, Nos. 50-63, 120 mg; Fraction E, Nos. 64-102, 100 mg; and Fraction F, Nos. 103-122, 50 mg.

Fraction A (712 mg) on gradient flash chromatography gave alstonidine (identified by uv, ir, ^{1}H nmr) (4 mg) and, after extensive further column and layer chromatography, a new alkaloid (4 mg), identified as 14-ketoalstonidine [**1**]. This compound was labile and could not be crystallized. It gave a yellow color with ceric ammonium sulfate and a positive Dragendorff test: uv (EtOH) λ max 226, 292, 376 nm (ε=20,400, 10,000, and 3800); ir ($CHCl_3$) ν max 3220, 1720, 1695, 1635 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 8.45 (1H, d, J=5.04 Hz), 8.22 (1H, d, J=5.03 Hz); 8.18 (1H, bd, J=7.83 Hz); 7.87 (1H, s); 7.67 (1H, d, d, d, J=8.3, 7.83 and 1.3 Hz); 7.52 (1H, bd, J=8.4 Hz); 7.38 (1H, m, J=8.4, 8.3 and 1 Hz); 5.08 (1H, d, J=4.9 Hz); 4.63 (1H, oct, J=10, 6.3 Hz); 3.8 (1H, dd, J=11.7, 4.2 Hz); 3.71 (3H, s); 3.52 (3H, s); 3.35 (1H, dd, J=11.7, 11.7); 2.23 (1H, m) and 1.4 (3H, d, J=6.39 Hz); ms *m/z* 394 (M^+, 65), 377 (19), 363 (36), 335 (17), 331 (18), 293 (15), 209 (18), 181 (100). Hrms gave $[M]^+$ at 394.15329 (calcd for $C_{22}H_{22}N_2O_5$, 394.15288).

Flash column chromatography of intermediate fractions gave 8 mg of chromatographically pure vincamedine.

Fractional crystallization of fraction B yielded alstonidine (300 mg) as colorless needles, mp 183°. After crystallization of alstonidine, the mother liquor was evaporated and the residue (1.0 g) separated into 88 fractions by flash column chromatography (4 cm column, 30-ml fractions) using $CHCl_3$-MeOH (19:1). From fractions 8-10 material (20 mg) was identified as 1-carbomethoxy-β-carboline (identified by uv, ir, ^{1}H nmr).

Further fractions from the preceding chromatogram of fraction B were flash chromatographed to give a major alkaloid, *O*-3,4,5-trimethoxybenzoylquebrachidine (identified by uv, ir, ^{1}H nmr), contaminated with minor amounts of other dihydroindole alkaloids.

Fractional crystallization of fraction C yielded a crystalline solid (150 mg). Tlc indicated the presence of some other Dragendorff-positive impurities, which were removed by flash column chromatography (2 cm, 10 ml fractions) using CH_2Cl_2-Me_2CO (1:4). This yielded a pure crystalline alkaloid (100 mg) mp 276°, identified as quebrachidine (by comparison with authentic material).

Fractions D, E, and F contained highly polar material which underwent decomposition very quickly on tlc plates during attempted purification. This prevented further investigation.

Partial Separation of $CHCl_3$ Extract.—All the $CHCl_3$-soluble alkaloids were very polar, and attempts to purify any alkaloid from the mixture always gave rise either to decomposition products or to very small amounts of unknown alkaloids which could not be identified. The only two compounds that could be separated and identified in a pure state were 1-carbomethoxy-β-carboline, which had been separated previously from the Et_2O-soluble extract, and 3,4,5-trimethoxybenzamide.

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