DNA-BASED ISOLATION AND THE STRUCTURE ELUCIDATION OF THE BUDMUNCHIAMINES, NOVEL MACROCYCLIC ALKALOIDS FROM ALBIZIA AMARA

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Abstract - On the basis of DNA affinity, a novel isolate was obtained from an extract prepared from the seeds of Albizia amara. As determined by a series of spectroscopic techniques, the isolate was structurally defined as a mixture of three macrocyclic alkaloids of the pithecolobine type that differed only in the length of the aliphatic side chain. The ^{1}H- and ^{13}C-nmr spectral parameters were unambiguously assigned to these alkaloids, which were given the trivial names budmunchiamine A, B or C. With the exception of former studies performed with Pithecolobium saman, this is the only other report of pithecolobine alkaloids being found in nature.

As a part of our ongoing effort to find new strategies for the discovery of biologically active compounds from natural sources, a rapid hplc-based method has recently been devised that detects compounds capable of binding to DNA.[1] This method is also applicable for the detection of binding agents in complex mixtures, such as plant extracts, and one of the

materials found to demonstrate a positive response with this detection system was an extract derived from the seeds of Albizia amara Bolv. (Leguminosae). This plant has previously been studied phytochemically, and a variety of compounds has been reported; examples include triterpenes,[2] flavonoids,[2,3] uncommon amino acids,[4] lipids,[5] and steroids.[6] None of these constituents, however, would be anticipated to yield a positive response in the DNA binding assay. We currently report the isolation and structure elucidation of the active principles.

The isolate (data are for the principal constituent) was obtained as a yellow oil with a molecular formula of $C_{27}H_{56}N_4O$ (hrms). It demonstrated no uv absorption spectrum, but did show absorption for an amide group (1638 cm^{-1}) in the ir spectrum. Parameters of the respective ^1H- and ^{13}C-nmr spectra obtained with the isolate are summarized in Table 1. The ^{13}C-nmr, APT and DEPT spectra displayed 24 distinct resonances, the intensity of one of these (at δ 29.6 ppm) corresponding to the remaining four carbon atoms. Using commonly accepted chemical shift correlation tables[7] for the assignment of functional groups, the nmr data presented above leads to the following preliminary structural inferences: the molecule contains 27 carbon atoms, as one C-methyl [(δ 13.5) in an aliphatic chain], three N-methyl (δ 35.3, 42.5 and 43.1), 14 C-CH_2 groups (mostly in a side chain), 5 N-CH_2 groups (δ 51.9, 55.0, 56.2, 56.6 and 56.8), two N-$CHCH_2$ (δ 29.8, 37.5), one CONH-CH_2 (δ 38.0), one N-CH (δ 61.4), and one amide carbonyl carbon atom (δ 172.5). Thus, these data suggested that the isolate might have a macrocycle component with a side chain.

Since many of the methylene signals were not well resolved, most of the ^1H-resonances were assigned to the corresponding carbon atoms on the basis of a hetero (^1H-^{13}C) correlation (HETCOR) map. Three low level cross-peaks were missing (at δ 51.9, 28.1 and 27.3), but they were readily reconstructed by using the one-dimensional ^1H-nmr spectrum and the complementary spin-connectivity information from the two-dimensional homo (^1H-^1H) correlation (COSY) map. The COSY spectrum demonstrated that the methine signal (H-4, δ 2.83) was coupled with one separated methylene signal at δ 2.23 and 2.27 (H-3), and a methylene signal at δ 1.25, which should be the terminal group of the side chain. This spectrum also demonstrated the presence of two chains each containing three methylene groups, and one chain of four methylene groups. In addition, it was found that the quartet observed at δ 3.32 (H-17) changed to a triplet when the triplet at 8.35 ppm was irradiated, demonstrating the spin-spin couplings between the two signals.

Selective INEPT experiments[8] permitted the unambiguous assignments of some carbon signals and also the characterization of the basic carbon skeleton. Thus, selective INEPT irradiation of H-4 (δ 2.83) enhanced C-2 (δ 172.5), the N_5-methyl (δ 35.3) and C-2' (δ 27.3), indicating that the side chain should be located at position C-4, and the amide group located at position C-3. Irradiation of H-6 (δ 2.62) enhanced C-4 (δ 61.4) and C-8 (δ 55.0) through three bonds,

and also C-7 (δ 26.8) through two bonds; these data position one of the chains containing three methylene groups between N_5 and N_9. Similarly, irradiation of H-17 (δ 3.32) enhanced the signals corresponding to C-2 and C-15 (δ 56.2), and also C-16 (δ 27.4) through two bonds. This serves to position a second chain containing three methylene groups between N_1 and N_{14}, and the remaining chain containing four methylene groups must be located between N_9 and N_{14}.

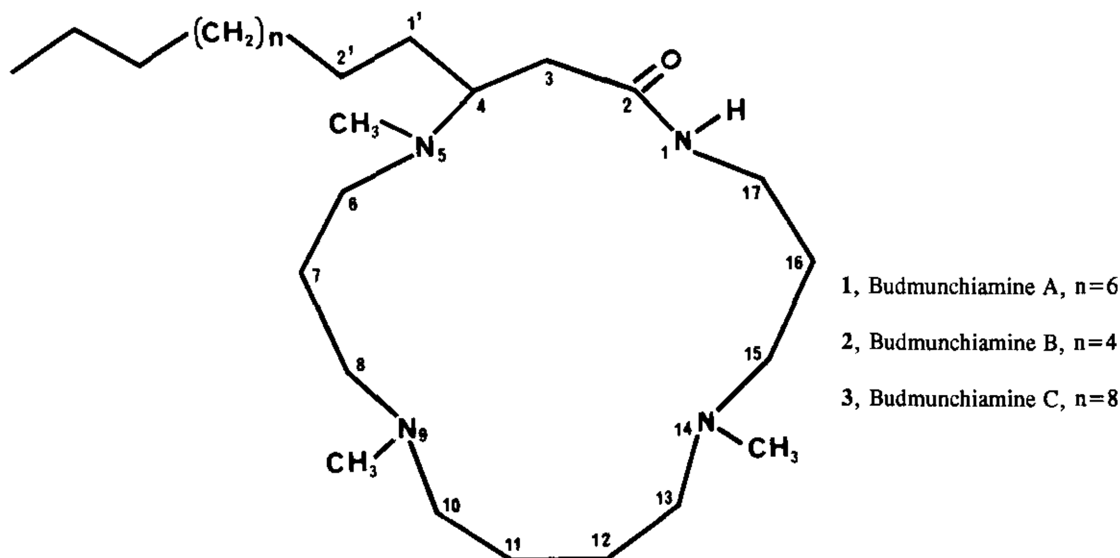
Table 1. ^{1}H and ^{13}C nmr Data Observed for the Alkaloid Preparation Isolated from Albizia amara.

Position	^{1}H (δ, ppm)	^{13}C (δ, ppm)
N_1H	8.35	----
C_2	----	172.5 s
C_3	2.27, 2.23 overlap	37.5 t
C_4	2.83 AMNXY	61.4 d
N_5CH_3	2.20 s	35.3 q
C_6	2.62, 2.40 ABt	51.9 t
C_7	1.61	26.8 t
C_8	2.38	55.0 t
N_9CH_3	2.22 s	43.1 q
C_{10}	2.5-2.2	56.8 t
C_{11}	1.52	24.8 t
C_{12}	1.54	23.9 t
C_{13}	2.5-2.2	56.6 t
$N_{14}CH_3$	2.18 s	42.5 q
C_{15}	~2.39	56.2 t
C_{16}	1.64	27.4 t
C_{17}	3.32 (q,7)	38.0 t
$C_{1'}$	1.25	29.8 t
$C_{2'}$	1.27 br	27.3 t
C_n	1.25	28.1, 29.3, 29.6
$C_{(n+2)'}$	1.25	31.9 t
$C_{(n+3)'}$	1.27 br	22.6 t
$C_{(n+4)'}$	0.88 (t,7)	13.5 q

The following summary is therefore consistent with these data: the isolate is a macrocycle containing 13 carbon atoms, one amide nitrogen atom, three additional nitrogen atoms at positions 5, 9 and 14, and one side chain at position C-4. In order to provide additional insight, the isolate was analyzed by high-resolution mass spectroscopy. The spectrum demonstrated a molecular peak with a mass of 452.44153 ($C_{27}H_{56}N_4O$, calcd 452.44539) and a significant peak was also observed at m/z 297, corresponding to $M^+ - C_{11}H_{23}$ (297.27173; $C_{16}H_{33}N_4O$, calcd 297.26543). This strongly supports the former suggestion of a macrocycle with a side chain linked in an α-position relative to an aliphatic amino-

group. Observation of the temperature-dependence of the mass spectrum revealed that peaks observed at m/z 424 and 480 belong to minor components of the preparation with an elemental composition corresponding to $\pm$ C_2H_4. The low intensity of the peaks at m/z 269 and 325 suggested that the structural difference among the homologues was present in the side chain.

Thus, in agreement with all of the nmr and ms data, it is concluded that the structure of the principal Albizia isolate is the macrocyclic compound 1.[9] Based on the mass spectral analysis, the side chain in the mixture varies in length and contains 9, 11 or 13 carbon atoms. The three homologues are present in the isolate in an approximate ratio of 1:4:1. A review of the literature indicates that this compound belongs to the class of pithecolobine alkaloids reported by Wiesner et al.[10] The current report is the first in which pithecolobine alkaloids have been characterized employing contemporary spectroscopic techniques, and in which the ^{1}H- and ^{13}C-nmr spectral parameters of this type of compound have been unambiguously assigned. It is notable that this spectroscopic approach of structure elucidation was suitable to delineate the composition of a mixture of homologues, the separation of which is not a trivial undertaking. Further, due to the methylation pattern within the macrocycle and the length of the side chain of one of the homologues (n=8), the molecular structures of these isolates are unique. In addition, this is the first report in which macrocyclic alkaloids of this type have been isolated from any plant other than Pithecolobium saman (syn. Samania saman).



The biological activity of these pithecolobine alkaloids is a further feature which has not previously been reported. Since we initially became interested in characterizing the structure of this substance due to its interaction with DNA, its biologic

potential is an obvious area of interest. In preliminary studies conducted with in vitro model systems, we have verified that the isolate does indeed interact with DNA with a K_D of approximately 2×10^{-5} M, and we have also found that it mediates a significant cytotoxic response with a number of cultured mammalian cells (ED_{50} values < 1 μg/ml). As judged by tests performed with Salmonella typhimurium strain TM677, the isolate is bactericidal at concentrations > 10 μg/ml, but is not mutagenic. We have also found that the isolate inhibits nucleic acid polymerases and platelet aggregation. A more complete description of the biological potential of these compounds will be described elsewhere.[11]

EXPERIMENTAL

Isolation of Budmunchiamines A-C. The seeds of Albizia amara Bolv. (Leguminosae) were collected in India in 1974 by staff members of the Economic Botany Laboratory, Agriculture Research Service, BARC-East, USDA, Beltsville, Maryland. Voucher specimens are on deposit at the herbarium of the National Arboretum, Washington, D.C. The seeds were milled, defatted with petroleum ether, and extracted at ambient temperature with MeOH to exhaustion. This extract was found to result in complete diminution of the DNA peak when evaluated by a recently described hplc elution technique.[1] The isolation procedure has been described in detail.[10] In brief, plant extract (6 mg) was applied to a column containing double-stranded DNA-cellulose (0.5 g, Sigma Chemical Co.), and the column was eluted essentially by the procedure of Zunino et al.[12] The isolate that was obtained on the basis of DNA affinity was examined by tlc using a variety of solvent systems, but development with $CHCl_3$:diethylamine (10:1) and/or cyclohexane:diethylamine (5:1) followed by detection with Dragendorff's reagent proved most valuable. These procedures revealed the presence of four spots (alkaloids), and these alkaloids were collected and used as markers to direct the isolation of larger quantities of the same substances from a total MeOH extract prepared from A. amara seeds. This extract (100 g) was dissolved in 2% aqueous HOAc (500 ml) and partitioned with $CHCl_3$ (500 ml). The aqueous acidic fraction was saved, and the $CHCl_3$ fraction was extracted with additional 2% aqueous HOAc (500 ml). The aqueous fractions were combined, adjusted to a pH of 9.0 with 30% NH_4OH, and extracted with $CHCl_3$ (1 l). The $CHCl_3$ fraction was recovered and concentrated to dryness. Using silica gel column chromatography with $CHCl_3$:diethylamine (150:1) as the solvent, this crude alkaloid fraction (21 g) was separated into three fractions. The first fraction was separated into two additional fractions by silica gel column chromatography with cyclohexane:diethylamine (50:1) as the solvent. One of these fractions (8 g) was used for the current study. When analyzed by tlc using $CHCl_3$:diethylamine (10:1) and/or cyclohexane:diethylamine (5:1) as the solvent system and Dragendorff's reagent for detection, the material appeared to a be homogeneous substance (single

spot) that was identical to one of the marker compounds that was obtained on the basis of DNA affinity using DNA-cellulose resin. Additional analysis by hplc using a C_{18} column (5μ particle size) failed to separate the isolate into multiple components.

Structure Elucidation of Budmunchiamines A-C. Infrared spectra were recorded in KBr pellet on a Nicolet MX-1 interferometer. ^{1}H-Nmr homonuclear COSY spectra and ^{13}C-nmr APT, DEPT and HETCOR spectra were recorded with a Bruker AC250 or a Nicolet NT360 spectrometer, in $CDCl_3$ solution, with TMS as an internal standard. Standard microprograms from the Bruker library were used. Selective INEPT experiments were recorded at 250/62.3 MHz or 360/90 MHz irradiating protons at 3.32, 2.83 and 2.62 ppm, according to the technique described by Bax.[13] Electron impact mass spectra (70 eV) were recorded with a Finnigan MAT-8430 mass spectrometer [accelerating voltage, 3 kV, ion-source temperature, 250° C, direct inlet, resolution 1250 (10% valley)], equipped with the SS300 data system.

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