NMR SPECTRAL ANALYSIS OF CADAMBINE FROM *ANTHOCEPHALUS CHINENSIS*

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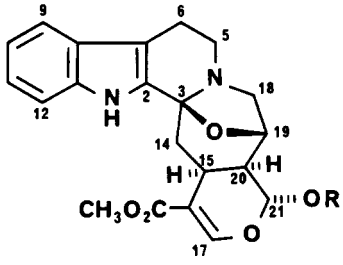
ABSTRACT.—The glycosidic monoterpenoid indole alkaloid cadambine (**1**) has been isolated in high yield from the leaves of *Anthocephalus chinensis* (Rubiaceae) and the structure confirmed by analysis of high-field proton and ^{13}C-nuclear magnetic resonance spectral data.

The genus *Anthocephalus* is a member of the tribe Naucleeae in the family Rubiaceae and forms the subtribe Anthocephalinae, being distinct from all other genera of the tribe. The alkaloids of the Naucleeae s.l. have been reviewed recently, and some 121 herbarium samples representing 18 genera of the tribe Naucleeae s.s. have been investigated for their alkaloid content (1).

The Indian tree *Anthocephalus chinensis* (Lamk.) A. Rich. ex Walp. (syn. *A. cadamba* Miq.) is distributed widely, from the lowlands of the Himalayas eastward to Assam and south through the western ghats to Karnatka and Kerala. Known as wild cinchona and popular in India as "Kadamb," its bitter and pungent bark is used in Ayurvedic medicine for uterine complaints, blood diseases, leprosy, and dysentery. A decoction of the leaves is recommended as a gargle in cases of stomatitis (2).

Little phytochemical work has been carried out on *A. chinensis*. Prasad and co-workers reported the presence of steroidal alkaloid constituents (3,4), and Brown and coworkers obtained the indole alkaloid cadambine (5), 3α-dihydrocadambine (5), isodihydrocadambine (6), cadamine (7), and isocadamine (7).

We now report the detection of alkaloids in all parts of *A. chinensis* (leaves 0.2%, fruits 0.05%, flowers 0.19%, and bark 0.18%) and the isolation,[3] from the leaves of this plant, of cadambine (**1**) in 0.1% yield. This alkaloid has recently been obtained from the bark of *Haldina cordifolia* (Roxb.) Ridsd. (syn. *Adina cordifolia* Hook.) (8) in 0.0067% yield, and some proton nmr data have been reported. We have now studied



1 R = glucose

2 R = glucose(Ac)$_4$

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the proton and ^{13}C-nmr spectra of cadambine (**1**) and cadambine tetra-acetate (**2**) and have made essentially complete assignments in both instances.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot plate and are uncorrected. The uv spectra were obtained with a Beckman model DB-G spectrophotometer, and ir spectra with a Beckman model IR-18A spectrophotometer with polystyrene calibration at 1601 cm^{-1}; absorption bands are reported in wave numbers (cm^{-1}). Proton nmr spectra were recorded at 270 MHz on a Brüker WH-270 instrument, and double irradiation experiments were performed at 200 MHz on a Varian XL-200 spectrometer. ^{13}C-nmr spectra were recorded at 22.68 MHz on a Brüker HFX-90 spectrometer. Mass spectra were obtained by using a Varian MAT 112S instrument, and circular dichroism data were determined with a Jasco 40A spectropolarimeter.

PLANT MATERIAL.—The leaves, bark, fruit, and flowers of *Anthocephalus chinensis* (Rubiaceae) were collected in September 1977 from trees cultivated on the campus of the Panjab University, Chandigarh, India, and were authenticated by the Curator, Department of Botany, Panjab University. An herbarium specimen has been deposited in the Department of Pharmaceutical Sciences, Panjab University, Chandigarh, India.

EXTRACTIONS AND PRELIMINARY FRACTIONATION.—Leaves of *A. chinensis* (7 kg) were dried, milled, and thoroughly extracted with hot ethanol (250 liters). After removal of the alcohol under reduced pressure, the residue (2.4 kg) was treated with 2% aqueous sulfuric acid, precipitated with Dragendorff's reagent, and the alkaloidal precipitates (380 g) were decomposed by the addition of silver carbonate. The bases were taken up in methanol, filtered, and evaporated to yield 59 g of crude alkaloids. Dissolution of the alkaloids in 2% aqueous sulfuric acid was followed by successive extractions with ether and chloroform, after which the solution was basified (pH 9) with concentrated ammonia.

ISOLATION OF CADAMBINE (**1**).—The precipitate formed by the addition of ammonia was filtered, dried, and chromatographed on neutral alumina eluted with methanol to yield cadambine (**1**, 6 g). Additional **1** was obtained as follows: the aqueous solution at pH 6.9 was successively extracted with ether (2 x 50 ml) and chloroform (10 x 50 ml), and the alkaloids were again precipitated with Dragendorff's reagent. After decomposition, as described previously, the crude alkaloid fraction (2.4 g) was repeatedly crystallized from methanol to afford **1** (1 g). The total cadambine isolated was 7 g, corresponding to a 0.1% yield.

PROPERTIES OF CADAMINE (**1**).—Cadambine, mp 208-210° [Lit. (5) 207-211°; Lit. (8) 220-223°]; $[\alpha]^{24}$ D −146° (c 0.112, MeOH) [Lit. (8) −115° (c 0.15, MeOH)]; ir, ν max (Nujol) 3600-3100 (H-bonded OH and NH), 1700 (unsaturated ester), and 1620 cm^{-1} (β-alkoxy unsaturated ester); uv, λ max (MeOH) (log ε) 230 (5.25), 240 (4.69), and 362 nm (4.38); λ max (MeOH + NaOH) 230 (5.25), 285 (4.17) and 293 nm (4.01); ^{1}H-nmr, see table 1; ^{13}C-nmr, see table 2; ms, *m/z* (%) 544 (M^+,6.9%), 381 (6.0), 380 (13.7), 365 (10.3), 364 (12), 344 (28), 321 (13.7), 262 (100), 254 (80), 233 (53), 225 (38), 199 (15), 192 (15), 185 (32), 184 (26), 170 (21), 169 (24), 168 (23), 156 (19), and 139 (36); cd, (c 0.0002, MeOH) $[\theta]_{291.5}$ −1,250, $[\theta]_{282.5}$ −1,500, $[\theta]_{234}$ −13,000, $[\theta]_{229}$ 0, and $[\theta]_{222}$ +13,000.

PREPARATION OF CADAMBINE TETRAACETATE (**2**).—Cadambine (**1**, 100 mg) was treated with acetic anhydride: pyridine (1:1, 4 ml) at room temperature for 18 h. Standard work-up procedures afforded 80 mg of **2**, crystallizing from ether-methanol, m.p. 148-150°; $[\alpha]^{24}$ D −122° (c 0.285, $CHCl_3$); ir, ν max (KBr) 3360 (br), 2950, 1750-1710, 1635, 1440, 1375, 1250 and 1165 cm^{-1}; uv, λ max (MeOH) (log ε) 230 (4.76), 240 (4.13), 270 (3.93), 285 (3.76) amd 362 nm (4.17), λ max (MeOH +NaOH) 230 (4.76), 270 (3.93) and 285 nm (3.75); ^{1}H-nmr, see table 1; ^{13}C-nmr, see table 2: ms, *m/z* (%) 712 (M^+, 6%), 382 (2), 381 (8), 365 (9), 364 (15), 332 (2), 331 (10), 321 (8), 271 (2), 254 (4), 253 (5), 225 (5), 212 (6), 211 (5), 199 (4), 187 (4), 186 (6), 185 (2), 184 (2), 170 (8), 169 (67), 158 (2), 157 (5), 139 (16), 127 (10), 115 (5), 109 (34), 83 (14), and 43 (100); cd (c 0.00024, MeOH) $[\theta]_{285}$ −4,170, $[\theta]_{241.5}$ −15,420, and $[\theta]_{229.5}$ 0.

NMR SPECTRAL ANALYSIS.—If the structure of cadambine as proposed by Brown (5,6) is correct, the 27 carbon atoms should appear as 7 singlets, 14 doublets, 5 triplets, and 1 quartet in the SFORD spectrum.

In practice, the SFORD spectrum of cadambine displayed all of these signals, and the multiplicities were in agreement with those anticipated. Many of the chemical shifts could be assigned by analogy with established data for compounds with a β-carboline nucleus (9) and with the nitrogenous glycosides related to strictosidine (10). Thus, the benzene nucleus and the methoxy carbonyl group were readily assigned and the remaining downfield methine (δ 152.35) could be attibuted to C-17 (10). The three remaining downfield quaternary carbons at δ 133.03, 109.68, and 109.19 could be assigned to C-2, C-7, and C-16, respectively (9,10), although the latter assignments might be reversed. No olefinic triplet or additional

TABLE 1. Proton nmr spectrum of cadambine tetra-acetate (**2**).[a]

Chemical Shift δ, ppm[b]	No. Protons	Multiplicity[c]	Assignment
1.658	1H	ddd	H-20
1.820	1H	t	H-14β
2.007	3H	s	-OCOCH$_3$
2.032	3H	s	-OCOCH$_3$
2.071	3H	s	-OCOCH$_3$
2.075	3H	s	-OCOCH$_3$
2.134	1H	dd	H-14α
2.675-2.860	2H	m	H$_2$-6′
2.957	1H	dd	H-18β
3.212	1H	dt	H-5′
3.291	1H	ddd	H-15
3.417	1H	d	H-18α
3.648	3H	s	-OCH$_3$
3.766	1H	dt	H-5α
4.230	1H	dd	H-6β
4.633	1H	dd	H-6α
4.809	1H	bd d	H-19
5.068-5.283	4H	m	H-1′, H-2′, H-3′, H-4′
5.190	1H	d	H-5β
5.661	1H	d	H-21
7.089	1H	t	H-11
7.166	1H	dt	H-10
7.335	1H	d	H-9
7.482	1H	s	H-17
7.535	1H	bd d	H-12
9.679	1H	bd	NH

Coupling constants: $J_{5\alpha,5\beta}$=9.6 Hz; $J_{5\alpha,6\alpha}$=3.0 Hz; $J_{5\alpha,6\beta}$=3.6 Hz; $J_{5\beta,6\beta}$=0 Hz; $J_{5\beta,6\alpha}$=0 Hz; $J_{6\alpha,6\beta}$=12.2 Hz; $J_{9,10}$=8.0 Hz; $J_{10,11}$=7.3 Hz; $J_{11,12}$=7.6 Hz; $J_{14\alpha,14\beta}$=12.6 Hz; $J_{14\alpha,15}$=5.6 Hz; $J_{14\beta,15}$=13.0 Hz; $J_{15,20}$=5.6 Hz; $J_{18\alpha,18\beta}$=10.3 Hz; $J_{18\alpha,19}$=0 Hz; $J_{18\alpha,19}$=7.1 Hz; $J_{19,20}$=1.7 Hz; $J_{20,21}$=9.3 Hz.

[a]Spectrum recorded at 270 MHz in $CDCl_3$.

[b]Values are in ppm (TMS=0).

[c]Multiplicities are given as s, singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; ddd, doublet of doublet of doublets; m, multiplet; bd, broad.

doublet for C-18 and C-19 in a vinyl group was observed, indicating that these carbons are involved in the skeletal modification. Carbons of the glucose unit were assigned by analogy with the iridoid loganin (10), although the resonances C-5′ and C-3′ could not be attributed unambiguously. A β-configuration at C-1′ was established (10).

Attention was then turned to the assignment of signals at δ 72.19 (d), 90.42 (s), and 100.18 (d). Significantly, no doublet (for C-3) was observed in the range δ 45-52 (10), indicating that this carbon was further substituted. The chemical shift of the remaining unassigned singlet (δ 90.42), which could be assigned to C-3, indicates substitution by oxygen (Δδ+43 ppm) (11). Carbon-21, which is substituted by two oxygen atoms, should be the most downfield doublet at δ 100.18, while the doublet at δ 72.19 should be the second terminal carbon of the ether linkage. Two triplets, at δ 51.80 and 21.48, were attributed to C-5 and C-6, respectively, by analogy with compounds in the yohimboid series (9). Thus, a triplet at δ 57.95 and a doublet at δ 24.88 remained to be assigned with two carbons not observed.

In order to complete the assignments and deduce the linkage of the ether residue, the spectrum of cadambine tetraacetate was examined. Most of the signals could be assigned by direct comparison with cadambine. Characteristic shifts were observed for acetylation of the sugar unit, which was established as glucose (12). Two additional carbons, previously under the umbrella of DMSO-d_6, now appeared at δ 43.35 (t) and 39.25 (d) and, with the triplet at δ 58.56 and the doublets at δ 72.64 and 25.92, could be assigned among C-14, C-20, C-18, C-19, and C-15, respectively.

TABLE 2. Carbon magnetic resonance chemical shifts for cadambine (**1**) and cadambine tetra-acetate (**2**).

Carbon	Cadambine (**1**)[a]	Cadambine Tetra-acetate (**2**)[b]
2	133.04	133.05
3	90.42	90.76
5	51.80	51.98
6	21.48	21.88
7	109.68[d]	110.94[f]
8	125.11	125.72
9	118.58	118.70[g]
10	118.58	119.08[g]
11	121.49	121.94
12	111.40	111.15
13	136.27	136.61
14	not observed[c]	43.35
15	24.88	25.92
16	109.19[d]	110.83[f]
17	152.35	152.15
18	57.95	58.56
19	72.19	72.64
20	not observed[c]	39.25
21	100.18	93.89
CO_2CH_3	166.43	166.87
CO_2CH_3	50.99	51.17
1′	96.30	95.51
2′	73.27	71.02
3′	76.94[e]	72.37[h]
4′	70.25	68.16
5′	76.56[e]	72.10[h]
6′	61.62	60.28
$CH_3C=O$	—	169.19, 170.00
$CH_3C=O$	—	20.42

[a]Recorded in DMSO-d_6.
[b]Recorded in $CDCl_3$.
[c]Overlap with DMSO-d_6 resonance.
[d-h]Assignments may be reversed.

Attachment of ether linkage to C-15 or C-20 would lead to a quaternary carbon and attachment at C-14 to an epoxide, which could specifically be eliminated (11). Because one of the methylenes adjacent to N-4 of the β-carboline remains to be assigned, it is apparent that C-18 is thus disposed, leaving C-19 as the only carbon available for oxygen substitution. The gross structure of cadambine is, therefore, confirmed to involve strictosidine, in which C-18 has cyclized to N-4 with an ether bridge linking C-3 and C-19, as proposed by Brown and Fraser (5).

Attention then focused on the proton nmr properties of cadambine tetraacetate (**2**) (table 1). Four acetyl methyl groups (δ 2.007, 2.032, 2.071, and 2.074), a methoxy carbonyl group (δ 3.647), four adjacent aromatic protons (δ 7.089, 7.166, 7.535), a singlet for H-17 (δ 7.482), and an indole NH (δ 9.769) could be readily assigned. More difficult to assign were the aliphatic protons of the nucleus and the glucose unit.

The simplest of the nuclear aliphatic signals was a sharp doublet (J=9.3 Hz) at δ 5.661 attributed to H-21, a methine proton on a carbon attached to two oxygen atoms. Irradiation at this frequency collapsed the signal at δ 1.658 to a doublet of doublets (J=1.7, 5.5 Hz) indicating it to be the *trans*-diaxial proton at C-20. Back irradiation at δ 1.658 re-emphasized this assignment and also simplified the signals at δ 3.291 (H-15) and δ 4.809 (H-19). The small coupling constant of 1.7 Hz observed between H-19 and H-20 is in agreement with a dihedral angle of ~60° between these two protons. Some sharpening of the region δ 2.13 was also observed suggesting the elimination of a long-range coupling.

Irradiation at δ 3.291 (H-15), besides simplifying H-20, revealed the signals at δ 1.820 and δ 2.134 to be the H-14 protons having a geminal coupling of 12.6 Hz. Assuming H-15 has the α configuration, as it does in all indole alkaloids derived from loganin (13), the former proton, with a $J_{14,\ 15\alpha}$ of 13.0 Hz,

could be assigned to H-14β, whereas the latter (J=5.6 Hz) could be attributed to H-14α. The magnitude of $J_{15,20}$ (5.6 Hz) indicates an angle of ~40° between these two protons. The remaining protons of the monoterpene unit could now be assigned by irradiation at δ 4.809 (H-19). Simplification was observed for the proton at δ 1.658 (H-20) to a doublet of doublets, the proton at δ 2.957 to a doublet and sharpening of the doublet at δ 3.417. These are, therefore, the two geminally coupled (J=10.3 Hz) H-18 protons adjacent to nitogen. Irradiation at δ 3.417 afforded a doublet (J=7.1 Hz) at δ 2.957, but hardly affected the doublet of doublets for H-19 at δ 4.809. Thus, H-19 and one of the H-18 protons subtend an angle close to 90° in the preferred conformation.

Four of the remaining protons to be assigned should be the H-5 and H-6 methylene protons. From the appearance of the signals at δ 4.230 and δ 4.633 as well-defined doublets of doublets, it was clear that these were the geminally coupled (J=12.2 Hz) H-6 protons, and this was confirmed through double irradiation. The complexity of the coupling indicated that each of these protons was principally coupled to only one of the adjacent methylene protons. However, since the signal at δ 3.766 appeared as a doublet of triplets, it was apparent that each of the H-6 protons must be coupling to the *same* H-5 proton. Dramatically, irradiation at δ 3.766 reduced the signals at δ 4.633 and δ 4.230 to a pair of doublets (J=12.2 Hz), and a doublet (J=9.6 Hz) at δ 5.190 to a singlet. This latter signal must, therefore, be the second H-5 methylene proton, surprisingly deshielded.

A typical *trans*-diaxial coupling constant of 9.3 Hz between H-20α and H-21β confirmed the stereochemistry of the glucose unit as α, and the small $J_{19,20}$ coupling constant indicated that H-19 had an α-configuration and that the oxygen bridge was β.

This same coupling constant also established the E-ring to exist in a chair conformation in agreement with the data observed for the H-14 protons. The configuration at C-3 was also evident from the strong negative rotation at 291, 282, and 234 nm in the cd spectrum of cadambine. A choice between an α- or a β-configuration for the nitrogen lone pair was made on the basis of the multiplicity and coupling constants of the 5- and 6- protons, since one of the protons appears as a doublet coupling with the doublet of triplets at δ 3.767. Dreiding models indicate this must be the 5β-proton in which little coupling is observed to either H-6α or H-6β. If the lone pair of N-4 is α, molecular models clearly indicate that the 5β-H and the 6α-H are almost *trans*-diaxial (=175°), whereas, when the N-4 lone pair is β, this angle is about 90°. Assignment of the 6α- and 6β-protons was made on the basis that irradiation of the 5α-H (δ 3.766 ppm) left a small, but observable, coupling between H-5β and the signal at δ 4.230. A distinct, positive nOe of 14% was also observed on the signal at δ 4,633, indicating that this was the 6α-H.

In summary, we believe that these data provide unambiguous proof of the structure and stereochemistry of cadambine (**1**), and also indicate that ring E exists in a chair conformation and that the N-4 lone pair and the ether oxygen both have the β-configuration.

DISCUSSION

Cadambine (**1**) was isolated previously from the bark of *A. chinensis* (5,6) and from the bark of *Haldina cordifolia* (8). Brown and co-workers had deduced the structure and stereochemistry of cadambine based on a number of chemical transformations including $NaBH_4$-reduction of cadambine tetra-acetate and spectral interpretation (5). Some double irradiation experiments were conducted, but relatively few assignments were made. Similarly, in the work of Cannon *et al.* (8) a number of proton assignments were made.

Our data confirm the structure of cadambine, including stereochemistry, and represent the first complete proton assignment of a compound in this series and the first attempt at assigning the ^{13}C-nmr spectrum. A number of the proton assignments made previously have been shown to be erroneous, and a very unusual downfield shift has been observed for an aliphatic proton adjacent to nitrogen.

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