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# ISOLATION OF CADAMBINE FROM *ADINA CORDIFOLIA* BENTH. AND HOOK. F.

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**Summary**

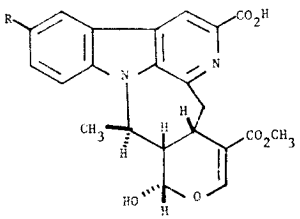
*Cadambine (5) has been isolated from the bark of* ***Adina cordifolia*** *Benth. and Hook. f.*

The genus *Adina* comprises 20 species which are distributed throughout tropical Africa and Asia[1]. No record has been found of the number of species which occur in Thailand, but *A. cordifolia* Benth. and Hook. f. is commonly encountered in Songkla province and is known locally as ข่าว ("kwow"). This species does not appear to be used in Thai folk remedies but according to Burkill[2] other *Adina* species have been claimed to have medicinal properties. In India, the wood of *A. cordifolia* has been used as a substitute for teak[2].

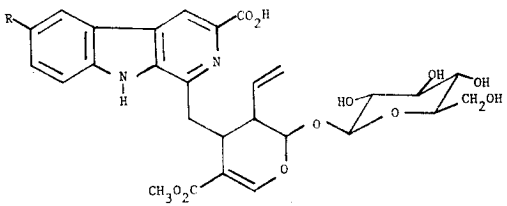
Following earlier work by Lal and Dutt[3], Cross *et al.*[4] isolated adifoline (1) from the wood of *A. cordifolia* and the structure (1) of this substance was established by Brown *et al.*[5] who also isolated cordifoline (3) from this species[6]. Merlini and Nasini[7] have since isolated 10-deoxyadifoline (2) and 10-deoxycordifoline (4) from the wood of *A. cordifolia.*

Both the fresh leaves and bark of *A. cordifolia* gave strongly positive field tests for alkaloids[8] and it was decided to investigate the bark of this species in the hope of obtaining further new bases. The conventional extraction procedure yielded a crude base which consisted mainly of a single crystalline substance which appeared to be a glycoside. Due to the low solubility of the base and to the decomposition which occurred at the melting point, satisfactory n.m.r. and mass spectra could not be obtained. For these reasons the substance was converted into the corresponding acetate which gave satisfactory spectra.

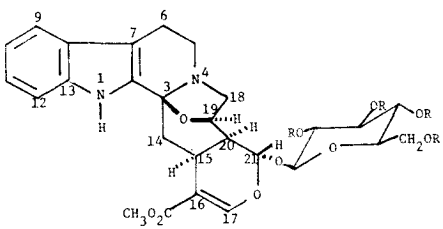
The acetate analysed for $C_{35}H_{42}N_2O_{15}$ but the molecular ion appeared at m/e 712 which corresponds with the formula $C_{35}H_{40}N_2O_{14}$; consequently, it appears that the acetate is a monohydrate. The properties of the tetraacetate were very similar to those recorded for cadambine tetraacetate (6) which was prepared from cadam-



(1) R = OH
(2) R = H



(3) R = OH
(4) R = H



(5) R = H
(6) R = Ac

bine (5), one of the alkaloids isolated from *Anthocephalus cadamba*, by Brown and Fraser[9]. Further work has confirmed that the two substances are, in fact, identical. Direct comparison of the two substances by Dr. R.T. Brown of the University of Manchester revealed that they had the same behaviour on t.l.c. in three solvent system, the n.m.r. spectra were identical, and the mixed m.p. was not depressed.

Although the rotation ($[\alpha]_D - 140.3^\circ$) of the base isolated in the present work is quite different from that recorded[9] for cadambine (5) ($[\alpha]_D - 71^\circ$) the C.D. curve showed a negative Cotton effect, in agreement with the absolute configuration preposed for C-3 in cadambine (5). Dr. Brown has suggested that the rotation of $-71^\circ$ is probably an error resulting from the use of an automatic polarimeter which has since been discovered to be faulty.

The occurrence of cadambine (5) in the bark of *A. cordifolia* is not surprising for this substance is related biogenetically to the other alkaloids which have been isolated from *Adina* species. In addition, *Adina* and *Anthocephalus* are considered to be closely related genera within the family Naucleaceae which is separated from the family Rubiaceae by some authors[1].

Microanalyses were carried out by the Australian Microanalytical Service, Melbourne. Melting points were determined on a Kofler hot stage. Rotations were measured with a Perkin Elmer 141 Polarimeter using a 1 dm microcell (1 ml) at room temperature. Infrared spectra were recorded with a Perkin Elmer 283 spectrophotometer. Low resolution mass spectra were measured on a Varian MAT-CH7 instrument using the conditions stated in each case. Only values for m/e greater than 100 and with a relative intensity greater than 10% of the base peak are usually quoted. Nuclear magnetic resonance (n.mr.) spectra were obtained with a Bruker HX90 spectrometer operating at 90MHz. Some signals were assigned and coupling constants were determined by means of nuclear magnetic double resonance techniques. Light petroleum refers to a fraction of boiling range 55–65°. A voucher specimen of the plant material has been deposited in the Herbarium, Department of Biology, Prince of Songkla University.

*Isolation of cadambine (5)*

Fresh bark (12kg) of *Adina cordifolia* Benth. and Hook. f. collected at Kuan Ka-long, Satul province, Southern Thailand in January 1976 was cut into small pieces and extracted with methanol at room temperature. The extract was evaporated under reduced pressure and the resulting brown-green tar (217g) was partitioned between ether (250 ml) and 2% aq. HCl (700ml). The acid layer was washed with ether (3 x 250ml) then basified (pH8) with aq. $NH_3$ and extracted with $CHCl_3$. Evaporation of the $CHCl_3$ gave the crude base (400mg). The aqueous layer was then adjusted to pH9 whereupon a dark brown tar was precipitated. This product was separated by decantation, washed with distilled water and taken up in a small volume of methanol. The solution was filtered to remove a brown precipitate (5.1g) which gave a negative test for alkaloids[8] then evaporated to yield a brown tar (18g), the t.l.c. of which was similar to that of the extract obtained at pH8.

The crude base (18g) was pre-adsorbed on silicic acid and chromatographed on a column of the same adsorbent (200g). The column was eluted with $CHCl_3$/MeOH (9:1) and 200ml fractions were collected. From the 7th to 10th fractions, inclusive, some crystals (400mg) were precipitated; a further quantity (1.4g) of crystalline material was obtained by evaporation of the filtrate and treatment of the residue (5g) with acetone (100ml) and water (25ml). The combined crystalline solid (1.8g) was repeatedly recrystallized from methanol to give cadambine (5) as colourless needles (800mg) which became pale yellow on storage; m.p. 220–223° (decomp. from 180°) (lit[9] m.p. 207–211°) $[\alpha]_D$ −140.3° (c, 0.27 in methanol) (lit[9] $[\alpha]_D$ −71° in methanol) (Found: C, 55.3; H, 6.1; N, 4.8%. Calc'd for $C_{27}H_{32}N_2O_{10}$: C, 59.6; H, 5.9; N, 5.1%; Calc'd for $C_{27}H_{32}N_2O_{10}.2H_2O$: C, 55.9; H, 6.3; N, 4.8%). C.D. $[\theta]$292: −10220 deg. $cm^2$ $decimole^{-1}$; $[\theta]$ 284: −11979 deg. $cm^2$ $decimole^{-1}$. N.m.r. spectrum (90MHz, $CF_3CO_2H$) $\delta$: 2.5–2.8, m, 1, CH; 3.2–4.9, m; 3.89, s, 3, $OCH_3$; 5.24, d (J 7Hz), 1, H1'; 5.94, d, (J 8Hz), 1, H21; 7.1–7.6, m, 4, H9, H10, H11, H12; 7.76, s, H17; 9.71 (br), s, 1, NH. Mass spectrum (160°/30eV) m/e: 542 ($M^+$–2H, 20%), 363 (15), 343 (25), 321 (15), 262 (100).

*Cadambine tetraacetate (6)*

The alkaloid (100mg) was treated with pyridine (2ml) and acetic anhydride (2ml) at room temperature overnight. Ice water was then added and the resulting solid was collected and crystallized from acetone/light petroleum to yield cadambine acetate (6) as silky needles (20mg) m.p. 146–150° (lit[9] m.p. 149–151°) $[\alpha]_D$ −115° (c, 0.15 in methanol) (lit[9] $[\alpha]_D$−93° in methanol). This substance had the same behaviour as authentic cadambine tetraacetate (6), $[\alpha]_D$ −132° on t.l.c. in three different solvent systems and the mixed m.p. was undepressed (R.T. Brown, personal communication). (Found: C, 57.6; H, 5.5; N, 3.9%. Calc'd for $C_{35}H_{40}N_2O_{14}$: C, 59.0 H, 5.7; N, 3.9%. Calc'd for $C_{35}H_{40}N_2O_{14}.H_2O$: C, 57.5; H, 5.8; N, 3.8%). N.m.r. spectrum (90MHz $CDCl_3$) $\delta$: 1.66, m, 1, H20; 1.97, 1.99, 2.03, 2.04, all s, 12, 4 × $CH_3CO$; 1.78–2.33, m, 2, H14; 2.82, s(br), 4, H5, H6; 2.94, m, 1, H18; 3.27 m, 1, H15; 3.48, m, 1, H18; 3.65, s, 3, $OCH_3$; 3.73, m, 1, H5'; 4.10–4.73, AB portion of ABX pattern, 2, H6'; 4.84, m, 1, H19; 5.00–5.31, m, 4; H1', H2', H3', H4'; 5.67, d (J9Hz), 1, H21; 7.00–7.63, m, 4 ArH; 7.48, s, 1, H17; 9.63, s(br), 1, NH. Mass spectrum (140°/30eV) m/e: 712 (30%), 381 (20), 364 (70), 331 (40), 321 (35), 271 (20), 254 (15), 212 (15), 169 (100), 109 (20).

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## บทคัดย่อ

จากการสกัดและตรวจหาโครงสร้างของสารในต้น *Adina cordifolia* Benth. & Hook. f. พบว่า มีแอลคาลอยด์ คือ cadambine