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THE ALKALOIDS OF *HOLARRHENA CURTISII* KING AND GAMBLE

JACK R. CANNON, EMIL L. GHISALBERTI and VITCHU LOJANAPIWATNA[a]

Department of Organic Chemistry, University of Western Australia, Nedlands, W.A. 6009, Australia

[a]*Present Address: Department of Chemistry, Faculty of Science, Prince of Songkla University, Haad-Yai, Songkla, Thailand.*

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Summary

The leaves of a Thai form of ***Holarrhena curtisii*** *King and Gamble have yielded the aminoglycosteroid holacurtine (2) and a new base which has been formulated as N–demethylholacurtine (4). The n.m.r. spectrum of N–acetylholacurtine (3) shows evidence of restricted rotation about the amide linkage but this feature is absent from the spectrum of N–acetyl–N–demethylholacurtine (5).*

Introduction

The genus *Holarrhena* belongs to the very important alkaloid-bearing family Apocynaceae and comprises 20 species which occur throughout Africa, Madagascar, India and Southeast Asia[1]. Although the Thai members of the genus have not yet been catalogued, three species—*H. antidysenterica* Wall, *H. densiflora* Ridl. and *H. curtisii* King and Gamble—are commonly found throughout the country[2]. Of these, *H. antidysenterica*, known locally as ไมกไหญ่ has been used in traditional Thai medicine for the treatment of dysentery[3]. In India this species has also been used for the same purpose[4].

The alkaloids isolated from *Holarrhena* species have been reviewed by Goutarel[5]. These substances fall into two distinct groups—the steroidal amines such as concurchine (1) and steroidal glycosides of an amino sugar. Holacurtine (2) is an example of the latter group. So far these aminoglycosteroids have only been isolated from three Asian *Holarrhena* species—*H. curtisii H. antidysenterica* and *H. mitis*[5].

Janot *et al*[6]. isolated holacurtine (2) and holacurtenine (6) from *H. curtisii* which had been collected in Malaysia and the plant was also found to contain a considerable amount of the aglycone holadiolone (7). These workers considered that holacurtenine (6) may be an artefact derived by dehydration of holacurtine (2). According to Goutarel[5] *H. crassifolia* which occurs in Kampuchea and Laos and which had been considered to be identical with the Malaysian *H. curtisii*, did not contain any ami-

noglycosteroids; instead concurchine (1) was the major constituent isolated from the leaves of this plant. From these findings it seemed that *H. curtisii* might be a variable species and as fresh leaves of this species collected near Trang gave a strongly positive field test for alkaloids[7], it was decided to investigate the constituents of this form.

Experimental

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 of m/e greater than 100 and with a relative intensity greater than 10% of the base peak are usually quoted. The 90MHz nuclear magnetic resonance (n.m.r.) spectra were obtained with a Bruker HX90 spectrometer; the 80 MHz Fourier transform n.m.r. spectra were measured with a Bruker WP80 instrument. 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 (PS74) of the plant material has been lodged in the Herbarium, Department of Biology, Prince of Songkla University, Haad-Yai.

*Extraction of **Holarrhena curtisii** King and Gamble*

Leaves were collected from plants growing near Trang, Southern Thailand, in February 1975. The milled, air-dried material (1.8kg) was extracted with methanol (2×5 l) at room temperature for 5 days then the combined extracts were concentrated to *ca* 1 l under reduced pressure and treated with ether (2 l) and 1% aq. HCl (1 l). The aqueous layer was extracted again with ether (2×1 l).

The combined ether extracts were washed with 1% aq. HCl, dried, passed through a column of charcoal (80g) then evaporated to yield a yellow oil (45.9g) which was then chromatographed on silicic acid (500g), but no holadiolone (7) could be detected in the eluate.

The acid extract was basified with conc. aq. NH_3 and the tarry mixture was extracted with chloroform. Evaporation of the solvent yielded a brown gum (7.8g) which was redissolved in 1% aq. HCl and the above process was repeated to give the crude base as a yellow gum (6.0g). Thin layer chromatography was carried out on a silicic acid plate using chloroform as the developing solvent in an atmosphere containing NH_3. The dried plate was sprayed with an iodoplatinate reagent; at least six compounds were present (R_F 0–0.28, 0.30, 0.38, 0.46, 0.56 and 0.66) but two of these (R_F 0.56 and 0.46) predominated. The crude base was then chromatographed on neutral alumina (activity 1, 180g) using varying proportions of benzene and chloroform to develop the column. Fractions (*ca.* 200ml) of the eluate were analysed by t.l.c. then combined and evaporated to yield three main fractions A, B, and C.

Examination of Fraction A

Fraction A (50mg) was eluted with benzene and t.l.c. revealed the presence of two bases R_F 0.66 and 0.56. The latter was apparently identical with holacurtine (2). The former (holacurtenine (6) ?) was separated by preparative layer chromatography and was obtained as a brown gum (10mg) which could not be induced to crystallize. Mass spectrum (110°/70eV) m/e: 473 (2%), 398 (9), 317 (10), 299 (42), 281 (8), 279 (9), 255 (10), 189 (8), 149 (70), 87 (100).

Examination of Fraction B—Isolation of Holacurtine (2)

Fraction B was eluted with benzene/chloroform (19:1); t.l.c. revealed the presence of one spot (R_F 0.56). The brown solid (1.40g) was dissolved in ether, washed with 1% aq. NaOH then extracted with 1% aq. HCl. The acid solution was basified with conc. aq. NH_3 and extracted with ether; evaporation of the solvent then gave a pale amorphous residue (1.1g). This product (200mg) crystallized with difficulty from ether/hexane to afford holacurtine (2) as needles m.p. 165–166° (lit[6] m.p. 162°) $[\alpha]_D$ +40.7° (c, 0.18 in chloroform) (lit.[6] $[\alpha]_D$ +42°). I.r. spectrum (Nujol) ν_{max}: 3300–3600cm^{-1} (br) (NH and OH), 1697cm^{-1} (s, CO). N.m.r. spectrum (90MHz, $CDCl_3$) δ: 0.80, s, 3, CH_3; 0.97, s, 3, CH_3; 1.00–2.20, m, *ca.* 24, steroidal H, H2' and H4'; 1.28, d, (J 6Hz), 3, CH_3; 2.21, s, 3, $COCH_3$; 2.40, s, 3, NCH_3; 2.90, m, 1, H17; 3.39, s, 3, OCH_3; 3.46–3.80, m, 3, H3, H3' and H5'; 4.34; s(br), 1, OH; 4.80, dd ($J_{1'a, 2'a} \sim 9.5$Hz; $J_{1'a, 2'e} \sim 2$Hz) 1, H1'. Mass spectrum (100°/70eV) m/e: 491 (4%), 416 (29), 317 (100), 299 (84), m* 282 (calc. 317 → 299, m* = 282), 281 (18), 177 (33), 158 (29), 87 (98).

Examination of Fraction C—Isolation of N–demethylholacurtine (4)

Although t.l.c. and n.m.r. suggested that fraction C (0.80g), which was eluted with benzene/chloroform (7:1), contained only one substance (R_F 0.46) attempts to crystallize it from several solvents failed. Moreover, each attempt at crystallization caused some decomposition for the colour of the product darkened. The fraction was redissolved in 1% aq. HCl and extracted with ether to remove coloured impurities then the aqueous solution was basified and extracted with ether. Evaporation of the solvent then gave a pale brown gum (700mg). This product (600mg) was extracted repeatedly with hot n-hexane; the insoluble brown solid (210mg) gave a faintly positive Mayers test. The combined cloudy extracts were evaporated and the colourless semi-solid residue (300mg) was repeatedly reprecipitated from ether/n-hexane to yield *N–demethylholacurtine* (4) as an amorphous solid (80mg) m.p. 135–141°. (Found: C, 67.7; H, 9.6; N, 2.6%. $C_{28}H_{47}NO_5$ requires C, 70.4; H, 9.9; N, 2.9%; $C_{28}H_{47}NO_5.H_2O$ requires C, 67.8; H, 10.0; N, 2.8%) $[\alpha]_D$ +24.7° (c, 0.2 in chloroform). I.r. spectrum (Nujol) ν_{max}: 3300–3630cm^{-1} (br, OH and NH): 1696cm^{-1} (s, CO). N.m.r. spectrum (80MHz, $CDCl_3$) δ: 0.81, s, 3, CH_3; 0.97, s, 3, CH_3; 1.0–2.2, m, *ca.* 24, steroidal H, H2' and H4'; 1.29, d (J 6Hz), 3, CH_3; 2.19, s, 3, $COCH_3$; 2.56, m, OH and NH; 2.90, m, 1, H17; 3.42, s, 3, OCH_3; 3.47–3.93, m, 3, H3, H3', H5'; 4.77, dd ($J_{1'a, 2'a} \sim 9.5$Hz, $J_{1'a, 2'e} \sim 2$Hz) 1, H1'. Mass spectrum 150°/30eV) m/e: 477 (3%) 433 (23), 405 (10), 373 (53), 317 (97), 299 (100), m* 282 (calc. 317 → 299, m* = 282) 281 (17). 177 (17), 144 (77), 143 (83), 73 (43).

N–acetylholacurtine (3)

Holacurtine (2) (60mg) in 1:1 aq. methanol (2ml) was treated with acetic anhydride (1ml) overnight. The solution was then diluted with water and extracted with ether. The extract was washed with 1% aq. HCl then dried and evaporated to yield a solid (30mg) which crystallized from acetone/light petroleum to give N–acetylholacurtine (3) as needles (15mg), m.p. 208–210° (lit.[6] m.p. 210–211°) $[\alpha]_D$ +56.4° (*c*, 0.3 in chloroform) (lit.[6] $[\alpha]_D$ +55°). N.m.r. spectrum (80MHz $CDCl_3$) δ: 0.78, s, CH_3; 0.97, s, CH_3, 1.0–2.2, m, steroidal H, H2'; 1.16, d (J 6Hz), CH_3; 1.22, d (J 6Hz), CH_3; 2.09, s, CH_3CON; 2.16, s, CH_3CON; 2.22, s, $CCOCH_3$; 2.88, s, NCH_3; 2.97, s, NCH_3; 3.31, s, OCH_3; 3.34, s, OCH_3; 3.40–4.47, m, H3, H3', H4' and H5'; 4.88, dd ($J_{1'a,2'a} \sim 9.5$Hz, $J_{1'a,2'e} \sim 2$Hz), H1'. (See Figure 1). Mass spectrum (110°/40eV) m/e: 533 (<1%), 431 (13), 317 (19), 299 (20), 200 (10), 168 (9), 129 (100).

N–acetyl–N–demethylholacurtine (5)

Crude N–demethylholacurtine (4) (100mg) was acetylated as above and the crude product (40mg) was subjected to preparative layer chromatography, using the rhodamine reagent to detect the band. This product crystallized from benzene/light petroleum to afford *N–acetyl–N–demethylholacurtine* (5) as plates (21 mg) m.p. 213–216°. (Found: C, 69.7; H, 9.3; N, 2,7. $C_{30}H_{49}NO_6$ requires C, 69.3; H, 9.5; N, 2.7%). $[\alpha]_D$ +22° (*c*, 0.1 in chloroform). I.r. spectrum (Nujol) ν_{max}: 3300–3600cm^{-1} (br, NH and OH); 1690cm^{-1} (m, CO); 1678cm^{-1} (s, CH_3CON). N.m.r. spectrum (80MHz, $CDCl_3$) δ: 0.79, s, 3, CH_3; 0.96, s, 3, CH_3; 1.0–2.2, m, steroidal H and H2'; 1.19, d (J 6Hz) 3, CH_3; 1.99, s. 3, CH_3CON; 2.22, s, 3, $CCOCH_3$; 2.90, m, 1, H17; 3.38, s, 3, OCH_3; 3.47–3.84, m, 4, H3, H3', H4' H5'; 4.80, dd ($J_{1'a,2'a} \sim 9.5$Hz, $J_{1'a,2'e} \sim 2$Hz) 1, H1'; 5.79, d ($J_{NH,4'a} \sim 9$Hz), 1, NH. (See Figure 2). Mass spectrum (100°/70eV) m/e 519 (absent), 475 (4%), 343 (3), 317 (6), 306 (4), 299 (14), 281 (4), 248 (8), 230 (6), 186 (15), 172 (13), 154 (35), 126 (14), 115 (100).

Results and Discussion

In the present work, chromatography of the neutral portion of an extract of the leaves yielded no holadiolone (7). However, t.l.c. of the crude basic fraction revealed the presence of at least six alkaloids; only two of these (R_F 0.56 and R_F 0.46) were present in significant amounts and both were isolated by extensive chromatography.

The physical properties of the major alkaloid with R_F 0.56 were found to be identical with those recorded for holacurtine (2) and in addition the m.p. and rotation of the acetyl derivative corresponded with those of N–acetylholacurtine (3)[6].

The second, minor, alkaloid with R_F 0.46 could not be obtained crystalline, but it has now been assigned the structure N–demethylholacurtine (4). This substance analysed for $C_{28}H_{47}NO_5.H_2O$ and it gave a crystalline N–acetyl derivative (5) which analysed for $C_{30}H_{49}NO_6$. The infrared spectrum of the base showed a broad absorption at 3300–3600cm^{-1}, attributed to the OH and NH_2 groups and the acetyl

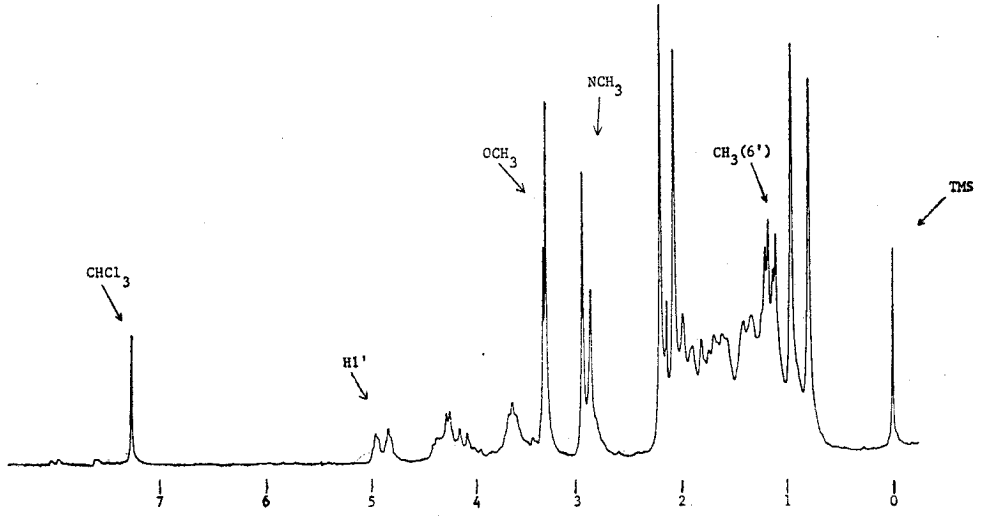


Fig. 1. N.m.r. spectrum (80MHz) of N–acetylholacurtine (3)

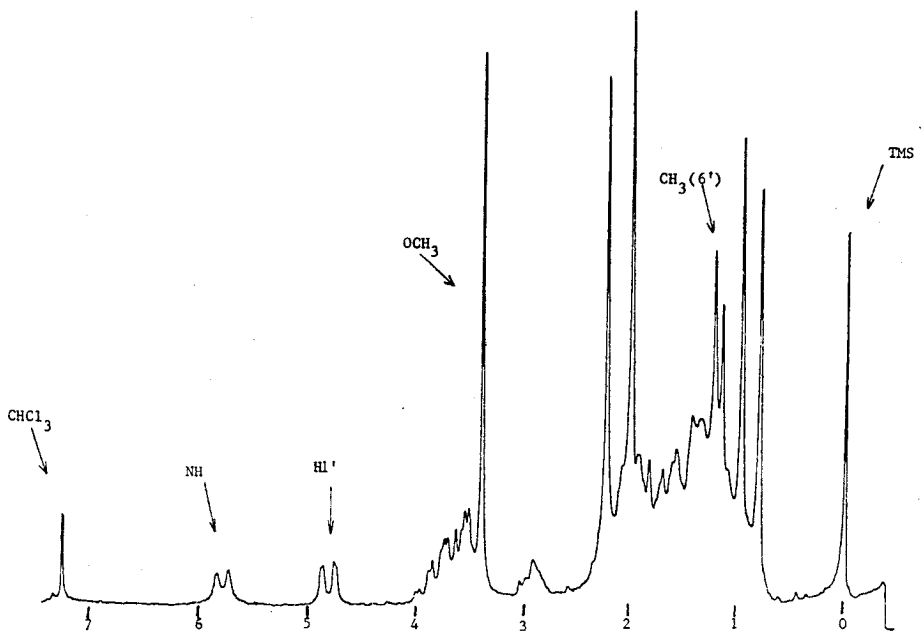
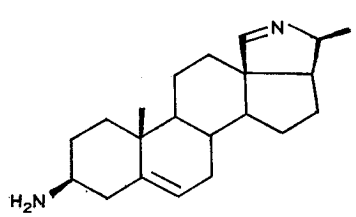
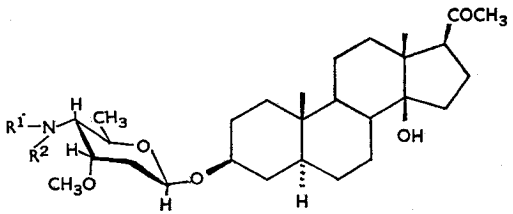


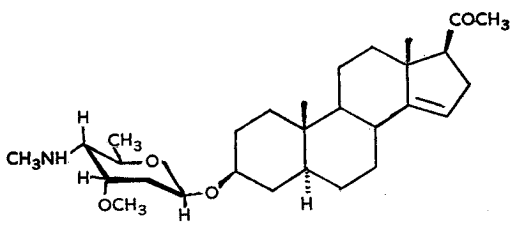
Fig. 2. N.m.r. spectrum (80MHz) of N–acetyl–N–demethylholacurtine (5)



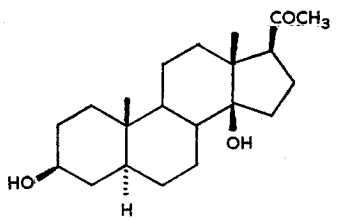
(1)



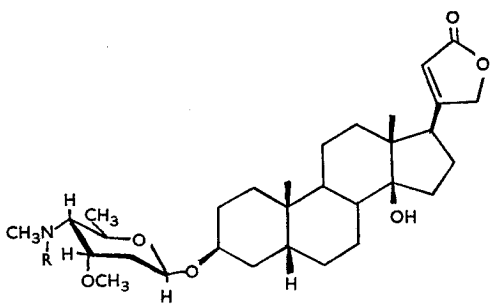
(2) $R^1 = H$, $R^2 = CH_3$
(3) $R^1 = Ac$, $R^2 = CH_3$
(4) $R^1 = H$, $R^2 = H$
(5) $R^1 = H$, $R^2 = Ac$



(6)



(7)



(8) R = H
(9) R = Ac

carbonyl gave rise to a band at 1696cm^{-1}. The mass spectrum of the base showed the molecular ion at m/e 477 i.e. 14 mass units lower than that of holacurtine (2). The remainder of the spectrum was similar to that of holacurtine (2)[8] with the exception of a characteristic peak at m/e 73 which was attributed to the ion $\overset{+}{H_2N}=CH-\dot{C}H-OCH_3$ from the aminosugar; holacurtine (2)[8] gave rise to an ion $CH_3\overset{+}{H}N=CH-\dot{C}H-OCH_3$ with m/e 87. The n.m.r. spectrum of N–demethylholacurtine (4) was also similar to that of holacurtine (2) with the exception of the singlet

at δ2.40 due to the N–methyl group of holacurtine (2) and the expected variation in the chemical shifts of the OH resonances.

Janot *et al*[6,9] did not record the n.m.r. spectra of N–acetylholacurtine (3) and N–acetylmitiphylline (9), which both contain the same N methylamino sugar residue. In the present work it was found that the n.m.r. spectrum of N–acetylholacurtine (3) (Fig. 1) contained double signals due to the C–methyl, N–methyl, acetyl and methoxy groups of the sugar residue. It seems that these may arise in the same way as those observed in the spectra of N,N–dimethylformamide and N,N–dimethylacetamide where apparently chemically equivalent methyl groups attached to the amide nitrogen become non-equivalent due to restricted rotation about the amide linkage as the result of contributions of charged forms to the resonance hybrid[10]. An attempt was made to test this hypothesis by carrying out a temperature dependent study of the n.m.r. spectrum of N–acetylholacurtine (3), but the double signals did not coalesce near the boiling point of $CDCl_3$.

Holacurtine (2) and mitiphylline (8) are the only N–methylaminoglycosteroid which have been isolated from *Holarrhena* species so far; the remaining aminoglycosteroids are all primary amines[5,11,12]. The n.m.r. spectra reported previously for the N–acetyl derivatives of the latter group show no doubling phenomena[11,12] and neither does the n.m.r. spectrum of N–acetyl–N–demethylholacurtine (5) (Fig. 2). Similarly, the n.m.r. spectra of N–methylacetamide and higher N–methylamides appear to arise from only one of the possible rotational conformers in which the NH and CO groups of the amide linkage have the *trans* configuration[10].

During the present work no evidence was obtained for the presence of a substantial amount of holacurtenine (6) in *H. curtisii*. Repeated chromatography of the crude bases gave only a trace of material with R_F 0.66 which may be identical with holacurtenine (6) for the mass spectrum of this product showed the molecular ion at m/e 473 with the base peak at m/e 87. The presence of a larger amount of this base would have been expected if, as suggested by Janot et al.[6], it is merely an artefact derived by dehydration of holacurtine (2).

The present work has confirmed that *H. curtisii* is a variable species and it has opened the way for a thorough investigation of specimens collected from different locations in Thailand.

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บทคัดย่อ

จากการสกัดและตรวจหาโครงสร้างของสารในใบของ *Holarrhena curtisii* King & Gamble (โมกใหญ่) ที่เก็บจากจังหวัดตรัง พบว่ามี แอลคาลอยด์ holacurtine (2) และ alkaloid ตัวใหม่ N–demethylholacurtine (4) จากการเปรียบ เอน เอ็ม อาร์ ของ N–acetylholacurtine (3) และ N–acetyl–N–demethylholacurtine (5) ปรากฏว่า ตัวแรกเกิด restricted rotation แต่ตัวหลังไม่เกิด