รายงานการวิจัย

การศึกษาสารกลุ่มอินโดลแอลคาลอยด์จากต้น *Alstonia glaucescens* (K. Sch.) Mona.

[Study on Indole Alkaloids from *Alstonia glaucescens* (K. Sch.) Mona.]

โดย

นาย นิวัติ แก้วประดับ

[Mr. Niwat Keawpradub]

รายงานการไปดูงานวิจัย ณ ประเทศญี่ปุ่นตามโครงการความร่วมมือทางวิชาการระหว่างสำนักงานคณะกรรมการวิจัยแห่งชาติ (NRCT) และองค์การส่งเสริมความก้าวหน้าทางวิทยาศาสตร์แห่งประเทศญี่ปุ่น (JSPS)

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บทคัดย่อ

การตรวจสอบเชิงพฤกษเคมีร่วมกับการตรวจหาฤทธิ์ทางชีวภาพของพืชนั้นถือได้ว่าเป็นวิธีมาตรฐานวิธีหนึ่งในการศึกษาวิจัยเพื่อหาตัวยาใหม่จากพืช จากการศึกษาเชิงพฤกษเคมีของพืชหลายชนิดในสกุล *Alstonia* วงศ์ Apocynaceae พบว่าสามารถแยกได้อินโดลแอลคาลอยด์ มากกว่า 130 ชนิดจากการวิจัยพืชสกุลนี้ 25 ชนิด ในขณะที่ยังไม่มีรายงานการศึกษาเชิงพฤกษเคมีของพืช *Alstonia glaucescens* (K. Sch.) Mona.

โครงการวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาองค์ประกอบทางเคมีโดยเฉพาะอย่างยิ่งสารกลุ่มอินโดลแอลคาลอยด์ที่แยกได้จากเปลือกต้นของพืช *Alstonia glaucescens* (K.

Sch.) Mona. โดยมีกระบวนการวิจัยเริ่มจาก การเก็บตัวอย่างพืชมาพิสูจน์เอกลักษณ์ทางพฤกษศาสตร์เพื่อให้ได้พืชตามชื่อวิทยาศาสตร์ที่ถูกต้อง แล้วเก็บรวบรวมเฉพาะส่วนของเปลือกต้นมาสกัดด้วยตัวทำละลายอินทรีย์แล้วนำสารสกัดหยาบที่ได้มาแยกให้บริสุทธิ์ด้วยวิธีการทางโครมาโตกราฟี ส่วนการพิสูจน์เอกลักษณ์โครงสร้างทางเคมีของสารบริสุทธิ์ที่แยกได้จะใช้เทคนิคทางสเปคโตรสโคปีเป็นสำคัญ

จากผงแห้งของเปลือกต้น *Alstonia glaucescens* (K. Sch.) Mona. จำนวน 2.2 กิโลกรัม สามารถสกัดได้สารสกัดหยาบของแอลคาลอยด์ 16.5 กรัม (7.5 กรัม/กิโลกรัม) และเมื่อนำมาแยกด้วย column chromatography, medium pressure liquid chromatography และ preparative thin-layer chromatography พบว่าได้สารบริสุทธิ์ 9 ชนิด โดยเป็นสารใหม่ในกลุ่มอินโดลแอลคาลอยด์ 3 ชนิด คือ echitaminic acid, 17-O-acetyl-N_b-demethylechitamine และ echitamidine N-oxide ที่เหลือเป็นสารเก่ากลุ่มอิริดอยด์ 1 ชนิด คือ sweroside และเป็นสารเก่ากลุ่มอินโดลแอลคาลอยด์ที่รู้สูตรโครงสร้างแล้ว 5 ชนิด คือ echitamidine, echitamine, N_b-demethylechitamine, 20-epi-19ξ-echitamidine, และ N_b-demethylechitamine N-oxide โดยในการพิสูจน์เอกลักษณ์สูตรโครงสร้างทางเคมีของสารบริสุทธิ์ที่แยกได้นี้ได้อาศัยเทคนิคทางสเปคโตรสโคปีขั้นสูงโดยเฉพาะอย่างยิ่ง 2D-NMR และ high resolution FAB-MS เป็นสำคัญ

อินโดลแอลคาลอยด์ที่แยกได้นี้สามารถใช้เป็นข้อมูลสนับสนุนในการจัดจำแนกหมวดหมู่ของพืชตามองค์ประกอบทางเคมี (Chemotaxonomy) และการศึกษากระบวนการเกิดชีวสังเคราะห์ (Biosynthesis) ของสารกลุ่มอินโดลแอลคาลอยด์ ของพืชในวงศ์ Apocynaceae ได้เป็นอย่างดี นอกจากนี้ควรจะได้นำอินโดลแอลคาลอยด์ที่แยกได้ใหม่ 3 ชนิดนี้ไปศึกษาฤทธิ์ทางชีวภาพเพื่อประเมินคุณค่าทางยาต่อไป

NRCT-JSPS Scientific Cooperation Program
under the Core University System

Subject of Research : Study on Indole Alkaloids from *Alstonia glaucescens* (K. Sch.) Mona.
[การศึกษาสารกลุ่มอินโดลแอลคาลอยด์จากต้น *Alstonia glaucescens* (K. Sch.) Mona.]

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ABSTRACT

The selection of plants having a specified class of chemical compound by phytochemical screening, followed by designated bioassay models is one of scientific methods for the selection of plants that can be expected to contain novel biologically active compounds. Phytochemical works of various species in the genus *Alstonia* of the family Apocynaceae are very interesting. More than 130 indole alkaloids have been isolated from 25 different species of this genus. While the plant *Alstonia glaucescens* (K. Sch.) Mona. is still not chemically screened for alkaloids.

The aim of this research work is to isolate and elucidate the structure of indole alkaloids from the stem bark of *Alstonia glaucescens* (K. Sch.) Mona. The research

strategy would proceed stepwise from selection and authentication of plant material, through collection, extraction, isolation and structure elucidation of the isolated compounds.

The crude alkaloids (16.5 g) were obtained by the acid-base extraction from the dried powdered stem bark of *Alstonia glaucescens* (K. Sch.) Mona. (2.2 kg). The yield of alkaloid extract was 7.5 g/kg. Three new indole alkaloids, echitaminic acid, 17-O-acetyl-N_b-demethylechitamine and echitamidine N-oxide along with one known iridoid compound, sweroside, and five known indole alkaloids namely, echitamidine, N_b-demethylechitamine, 20-epi-19ξ-echitamidine, N_b-demethylechitamine N-oxide and echitamine were separated from the alkaloid extract by means of column chromatography, medium pressure liquid chromatography and preparative thin-layer chromatography. Their structures were elucidted by full range of spectroscopic techniques especially 2D-NMR experiments and high resolution FAB-MS.

The presence of these indole alkaloids from this particular plant is an important supporting evidence for chemotaxonomy and biosynthetic study of chemical constituents from plants in the family Apocynaceae. The biological evaluation and chemical modification of the three new indole alkaloids remain of great intrinsic scientific interest for the further investigation.

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LIST OF ABBREVIATIONS

aq	aqueous
br	broad (for NMR spectra)
°C	degree Celsius
$CHCl_3$	chloroform
^{13}C NMR	carbon-13 nuclear magnetic resonance
d	doublet (for NMR spectra)
2D NMR	two dimensional nuclear magnetic resonance
EtOAc	ethyl acetate
EIMS	electron impact mass spectrometry
g	gram
^{1}H NMR	proton nuclear magnetic resonance
HR FAB MS	high resolution fast-atom bombardment mass spectrometry
IR	infrared
KBr	potassium bromide
kg	kilogram
m	meter
m	multiplet (for NMR spectra)
M^+	molecular ion
m/z	mass to charge ratio
MCPBA	meta-chloroperbenzoic acid
MeOH	methanol
mg	milligram
MHz	megahertz
ml	milliliter
mp	melting point

M_r	molecular weight
NH_3	ammonia
nm	nanometer
ppm	parts per million
q	quartet (for NMR spectra)
s	singlet (for NMR spectra)
t	triplet (for NMR spectra)
δ	chemical shift (ppm, for NMR spectra)
UV	ultraviolet

KEY WORD INDEX

Alstonia glaucescens (K. Sch.) Mona.

Apocynaceae

Stem bark

Indole alkaloids

17-O-Acetyl-N_b-demethylechitamine

Echitaminic acid

Echitamidine N-oxide

Echitamidine

20-epi-19ξ-echitamidine

N_b-demethylechitamine

N_b-demethylechitamine N-oxide

Echitamine

Sweroside

MATERIALS AND METHODS

Instruments: Mps were uncorrected; UV spectra were recorded in MeOH; IR as KBr discs; ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125.65 MHz, respectively (ppm, *J* in Hz with TMS as internal standard). EIMS were obtained by direct probe insertion at 70 eV. HR FAB-MS were recorded on a JEOL JMS-HX 110A spectrometer. CC: silica gel 60 (230-400 mesh); TLC: precoated Kieselgel 60 F_{254} (0.25 mm thick); Prep. TLC: silica gel GF_{254} (Merck 7730, 0.5 mm thick); MPLC: silica gel prepacked column Si-5 No. 05133.

Plant Material: The stem bark of *Alstonia glaucescens* (K.Sch.)Mona. was collected at Songkhla, Thailand in June 1993. The herbarium specimen has been deposited at Faculty of Pharmaceutical Sciences, Prince of Songkla University. The plant was directly compared with the standard specimen of *A. glaucescens* (K.Sch.)Mona. (No. 0002719), identified by K.M. Wong in 1986, deposited at the Herbarium of Faculty of Science, Prince of Songkla University.

Extraction and Isolation: The dried powdered stem bark (2.2 kg) of *A. glaucescens* was percolated with MeOH. The combined MeOH percolate was concentrated to syrupy mass and dissolved in 5 % aq. HOAc. The acidic filtrate was basified with aq. NH_3 and extracted with $CHCl_3$ at pH 9. Drying and evaporation yielded 16.5 g of crude alkaloid extract. An aliquot (10.0 g) was chromatographed on silica gel column eluting with 0-100 % MeOH-$CHCl_3$ to give 5 frs. Fr. A (1.3 g) was separated by CC eluting with 0-20 % MeOH-$CHCl_3$ followed by prep. TLC (aq. NH_3-sat. $CHCl_3$) to give **1** (68 mg) and by prep. TLC (8 % MeOH-$CHCl_3$) to yield **4** (4 mg). Fr. B (2.0 g) was subjected to CC eluting with 20 % MeOH-EtOAc to give **5** (350 mg). Fr. C (3.2 g) was subjected to CC eluting with 15-70 % MeOH-EtOAc to yield **5** (760 mg). The remaining mixture was further purified by MPLC using 40 %

MeOH-EtOAc as solvent to give **2** (110 mg). Fr. D (1.4 g) was fractionated by CC with 20-100 % MeOH-EtOAc into 3 frs (D1-3). Fr. D1 was purified by prep. TLC (15 % MeOH-$CHCl_3$) to give **3** (33 mg). Fr. D2 was purified by MPLC (18 % MeOH-EtOAc) to yield **9** (6 mg). Fr. D3 was purified by MPLC (50 % MeOH-EtOAc) to give **6** (21 mg). Fr. E (1.2 g) was chromatographed on CC eluting with 40-100 % MeOH-EtOAc followed by MPLC (60 % MeOH-EtOAc) to give **8** (26 mg). The remaining mixture was further purified by CC eluting with 60-100 % MeOH-$CHCl_3$ to yield **7** (84 mg).

Structure Elucidation of the Isolated Compounds:

Compound **1** (echitamidine). Amorphous, UV λ_{max} nm: 330, 296, 235; IR ν_{max} cm^{-1}: 3420, 3350, 1665, 1600; EIMS *m/z* (rel. int.) : 340 $[M]^+$ (19), 296 (5), 241 (100), 225 (11), 180 (33) 139 (10), 105 (3); ^1H NMR (500 MHz, $CDCl_3$): δ 8.64 (1H, br s, NH), 7.19 (1H, br d, *J*=7.6 Hz, H-9), 7.15 (1H, td, *J*=7.6, 1.0 Hz, H-11), 6.93 (1H, td, *J*=7.6, 1.0 Hz, H-10), 6.85 (1H, br d, *J*=7.6 Hz, H-12), 3.91 (1H, br s, H-3), 3.88 (3H, s, OMe), 3.33 (1H, br d, *J*=1.7 Hz, H-15), 3.27 (1H, dq, *J*=11.8, 6.1 Hz, H-19), 3.10 (1H, m, H-5), 2.91 (1H, dd, *J*=11.4, 4.3 Hz, H-21), 2.87 (1H, dd, *J*=13.0, 1.6 Hz, H-5), 2.82 (1H, m, H-6), 2.04 (1H, ddd, *J*=13.0, 3.0, 1.8 Hz, H-14), 1.94 (1H, br t, *J*=11.4 Hz, H-21), 1.86 (1H, m, H-6), 1.77 (1H, m, H-20), 1.42 (1H, ddd, *J*=13.0, 3.0, 2.0 Hz, H-14), 1.16 (3H, d, *J*=6.1 Hz, H-18); ^{13}C NMR: Table 1.

Compound **2** (20-epi-19ξ-echitamidine). Amorphous,UV λ_{max} nm: 328, 297, 228; IR ν_{max} cm^{-1}: 3430, 3350, 1665, 1605; EIMS *m/z* (rel. int.): 340 $[M]^+$ (39), 296 (4), 241 (21), 225 (100), 208 (37), 180 (84), 139 (42), 94 (71), 55 (62); ^1H NMR (500 MHz, $CDCl_3$): δ 8.51 (1H, br s, NH), 7.21 (1H, br d, *J*=7.6 Hz, H-9), 7.15 (1H, td, *J*=7.6, 1.2 Hz, H-11), 6.91 (1H, td, *J*=7.6, 1.2 Hz, H-10), 6.85 (1H, br d, *J*=7.6 Hz, H-12), 4.04 (1H, br d, *J*=1.7 Hz, H-3), 3.84 (3H, s, OMe), 3.57 (1H, dq, *J*=12.0, 6.1 Hz, H-19), 3.21 (1H, ddd, *J*=11.7, 7.3, 4.2 Hz, H-5), 3.05 (1H, ddd,

J=11.7, 7.1, 4.4 Hz, H-5), 2.95 (1H, dd, *J*=5.0, 2.7 Hz, H-15), 2.88 (1H, dd, *J*=14.4, 12.2 Hz, H-21), 2.63 (1H, dd, *J*=14.4, 6.1 Hz, H-21), 2.29 (1H, m, H-6), 2.26 (1H, m, H-14), 1.98 (1H, ddd, *J*=12.7, 6.5, 4.4 Hz, H-6), 1.79 (1H, m, H-20), 1.19 (1H, ddd, *J*=13.6, 2.8, 2.0 Hz, H-14), 1.12 (3H, d, *J*=6.1 Hz, H-18); ^{13}C NMR: Table 1.

Compound **3** (echitamidine N-oxide). Needles from EtOAc-MeOH, mp 187-188°C, UV λ_{max} nm : 329, 292, 232; IR ν_{max} cm^{-1}: 3425, 3355, 1665, 1602; EIMS *m/z* (rel. int.): 356 $[M]^+$ (3), 340 $[M-16]^+$ (23), 296 (9), 281 (4), 241 (57), 225 (17), 194 (16), 180 (21), 94 (31), 69 (100); HR FAB MS *m/z* found 357.1809 $[M+H]^+$, calc. for $C_{20}H_{25}N_2O_4$: 357.1808; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.36 (1H, s, NH), 7.45 (1H, br d, *J*=7.1 Hz, H-9), 7.14 (1H, td, *J*=7.6, 1.2 Hz, H-11), 7.01 (1H, br d, *J*=7.6 Hz, H-12), 6.86 (1H, td, *J*=7.1, 0.9 Hz, H-10), 4.06 (1H, br s, H-3), 3.73 (3H, s, OMe), 3.50 (1H, dt, *J*=12.8, 7.9 Hz, H-5), 3.37 (1H, br d, *J*=13.0 Hz, H-5), 3.32 (1H, br s, H-15), 3.26 (1H, dq, *J*=10.4, 6.0 Hz, H-19), 3.16 (1H, br t, *J*=10.5 Hz, H-21), 3.01 (1H, dd, *J*=11.9, 4.1 Hz, H-21), 2.52 (1H, m, H-6), 2.28 (1H, ddd, *J*=13.0, 3.8, 1.8 Hz, H-14), 2.06 (1H, m, H-20), 1.79 (1H, dd, *J*=13.8, 7.5 Hz, H-6), 1.18 (1H, ddd, *J*=13.0, 4.0, 2.0 Hz, H-14), 1.01 (3H, d, *J*=6.4 Hz, H-18); ^{13}C NMR: Table 1.

MCPBA oxidation of 1 to 3 : To a soln of **1** (10 mg : 0.03 mmol) in dry CH_2Cl_2 (0.4 ml), MCPBA (5.5 mg) was added and stirred at 0° for 15 min. After the addition of aq. NH_3 (4 ml), the whole was extracted with 5 % MeOH-$CHCl_3$. The residue showed one spot on TLC. After crystallization, a pure compound (needles, 6 mg) was obtained, which was identical with natural N-oxide (**3**) on TLC, UV, EIMS and ^{1}H NMR.

Compound **4** (17-O-acetyl-N$_b$-demethylechitamine). Amorphous, UV λ_{max} nm: 299, 243, 205; EIMS *m/z* (rel. int.): 412 $[M]^+$ (41), 395 $[412-OH]^+$ (35), 353 (10), 281 (4), 167 (10), 57 (100); HR FAB MS *m/z* found 413.2075 $[M+H]^+$, calc. for $C_{23}H_{29}N_2O_5$: 413.2069; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.69 (1H, dd, *J*=7.6, 1.2 Hz, H-9), 7.00 (1H, td, *J*=7.6, 1.2 Hz, H-11), 6.68 (1H, td, *J*=7.6, 1.2 Hz, H-10), 6.45

(1H, br d, *J*=7.8 Hz, H-12), 5.41 (1H, br q, *J*=6.6 Hz, H-19), 4.79 (1H, d, *J*=12.0 Hz, H-17), 4.28 (1H, dd, *J*=10.8, 5.7 Hz, H-3), 4.21 (1H, br d, *J*=16.2 Hz, H-21α), 3.79 (3H, s, OMe), 3.77 (1H, d, *J*=5.2 Hz, H-15), 3.73 (1H, d, *J*=12.0 Hz, H-17), 3.40 (1H, dt, *J*=10.7, 7.4 Hz, H-5), 3.01 (1H, d, *J*=16.0 Hz, H-21β), 2.75 (1H, dd, *J*=11.8, 8.3 Hz, H-5), 2.46 (1H, ddd, *J*=15.0, 10.0, 5.4 Hz, H-14β), 2.18 (1H, br dt, *J*=15.0, 8.3 Hz, H-6), 2.07 (1H, dd, *J*=13.8, 7.4 Hz, H-6), 2.06 (3H, s, OAc-Me), 1.77 (3H, dd, *J*=7.0, 2.2 Hz, H-18), 1.64 (1H, ddd, *J*=15.0, 5.1, 1.0 Hz, H-14α); ^{13}C NMR: Table 1.

Acetylation of 5 to 4 : Upon acetylation of **5** (10 mg) with pyridine and 1 eq of Ac_2O at room temp. for 48 hrs., and after purification by prep. TLC, this reaction give 1 mg of compound which was identical with **4** on TLC, EIMS and ^{1}H NMR.

Compound **5** (N_b-demethylechitamine). Needles from MeOH, mp 215-217°C, UV λ_{max} nm: 299, 241, 204; IR ν_{max} cm^{-1}: 3398, 3300, 1742, 1605; EIMS *m/z* (rel. int.): 370 $[M]^+$ (52), 353 $[370\text{-}OH]^+$ (49), 267 (9), 154 (19), 130 (55), 81 (38), 55(100); ^{1}H NMR (500 MHz, $CDCl_3$-CD_3OD): δ 7.57 (1H, dd, *J*=7.6, 1.2 Hz, H-9), 6.98 (1H, td, *J*=7.6, 1.2 Hz, H-11), 6.64 (1H, td, *J*=7.6, 1.2 Hz, H-10), 6.42 (1H, dd, *J*=7.6, 1.2 Hz, H-12), 5.38 (1H, q, *J*=6.6 Hz, H-19), 4.27 (1H, dd, *J*=10.9, 5.5 Hz, H-3), 4.23 (1H, br d, *J*=16.0 Hz, H-21α), 4.04 (1H, d, *J*=12.0 Hz, H-17), 3.83 (1H, *J* partly overlapped with δ 3.82, H-15), 3.82 (3H, s, OMe), 3.40 (1H, dt, J=11.8, 7.0 Hz, H-5), 3.36 (1H, d, *J*=12.0 Hz, H-17), 2.96 (1H, d, *J*=16.0 Hz, H-21β), 2.74 (1H, dd, *J*=11.8, 7.6 Hz, H-5), 2.58 (1H, ddd, *J*=14.9, 10.9, 5.4 Hz, H-14β), 2.24 (1H, dt, *J*=13.7, 7.5 Hz, H-6), 2.07 (1H, dd, *J*=13.7, 7.6 Hz, H-6), 1.73 (3H, dd, *J*=7.1, 2.2 Hz, H-18), 1.59 (1H, ddd, *J*=14.9, 5.8, 1.3 Hz, H-14α); ^{13}C NMR: Table 1.

Compound **6** (N_b-demethylechitamine N-oxide).Needles from MeOH, UV λ_{max} nm: 298, 240, 203; FAB MS *m/z* : 387 $[M+H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$-CD_3OD): δ 7.51 (1H, br d, *J*=7.8 Hz, H-9), 7.04 (1H, br t, *J*=7.3 Hz, H-11),

6.67 (1H, br t, *J*=7.7 Hz, H-10), 6.64 (1H, br d, *J*=7.8 Hz, H-12), 5.66 (1H, br q, *J*=6.5 Hz, H-19), 4.73 (1H, dd, *J*=14.1, 2.0 Hz, H-21α), 4.47 (1H, dd, *J*=10.9, 5.0 Hz, H-3), 4.03 (1H, d, *J*=15.1 Hz, H-21β), 3.95 (1H, d, *J*=4.9 Hz, H-15), 3.84 (1H, d, *J*=12.2 Hz, H-17), 3.80 (3H, s, OMe), 3.77 (1H, br dd, *J*=10.2, 2.4 Hz, H-5), 3.25 (1H, d, *J*=12.2 Hz, H-17), 3.23 (1H, dd, *J*=10.2, 7.6 Hz, H-5), 2.67 (1H, ddd, *J*=15.3, 10.7, 5.4 Hz, H-14β), 2.36 (1H, dt, *J*=14.7, 7.0 Hz, H-6), 2.10 (1H, dd, *J*=14.7, 8.5 Hz, H-6), 1.74 (3H, dd, *J*=7.1, 2.2 Hz, H-18), 1.73 (1H, *J* partly overlapped with δ 1.74, H-14α); ^{13}C NMR: Table 1.

Compound **7** (echitamine). Crystals from MeOH, mp 285-289°C (dec.), UV λ_{max} nm: 294, 236, 207; IR ν_{max} cm^{-1}: 3405, 3200, 1735, 1600; EIMS *m/z* (rel. int.): 384 $[M]^+$ of Hofmann base (40), 252 (13), 232 (34), 194(15), 152 (39), 58 (100); FAB MS *m/z* : 385 (100); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.74 (1H, dd, *J*=7.6, 1.0 Hz, H-9), 7.61 (1H, br s, NH), 7.10 (1H, td, *J*=7.6, 1.0 Hz, H-11), 6.75 (1H, td, *J*=7.6, 1.0 Hz, H-10), 6.73 (1H, dd, *J*=7.6, 1.0 Hz, H-12), 5.73 (1H, q, *J*=6.4 Hz, H-19), 4.42 (1H, br d, *J*=14.9 Hz, H-21α), 4.36 (1H, dd, *J*=10.6, 5.5 Hz, H-3), 4.25 (1H, d, *J*=14.9 Hz, H-21β), 3.86 (1H, d, *J*=4.7 Hz, H-15), 3.74 (1H, d, *J*=10.2 Hz, H-17), 3.73 (3H, s, OMe), 3.63 (1H, dd, *J*=12.7, 8.5 Hz, H-5), 3.37 (1H, m, H-5), 3.29 (3H, s, N-Me), 3.16 (1H, d, *J*=10.2 Hz, H-17), 2.59 (1H, ddd, *J*=15.0, 10.6, 5.5 Hz, H-14β), 2.24 (1H, dt, *J*=14.2, 8.5 Hz, H-6), 2.02 (1H, dd, *J*=14.2, 8.4 Hz, H-6), 1.79 (3H, dd, *J*=6.4, 1.5 Hz, H-18), 1.52 (1H, ddd, *J*=15.0, 5.9, 1.0 Hz, H-14α); ^{13}C NMR: Table 1.

Compound **8** (echitaminic acid). Crystals from MeOH, mp 218-220°C, UV λ_{max} nm: 291, 236, 205; IR ν_{max} cm^{-1}: 3400, 3245, 1615, 1595; FAB MS (with KI) *m/z* : 409 $[M+K]^+$; HR FAB MS *m/z* : found 371.1967 $[M+H]^+$, calc. for $C_{21}H_{27}N_2O_4$: 371.1964; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.70 (1H, br d, *J*=7.5 Hz, H-9), 7.30 (1H, br s, NH), 6.99 (1H, br t, *J*=7.5 Hz, H-11), 6.68 (1H, br d, *J*=7.5

Hz, H-12), 6.64 (1H, br t, *J*=7.5 Hz, H-10), 5.66 (1H, q, *J*=6.6 Hz, H-19), 4.45 (1H, br d, *J*=4.1 Hz, H-3), 4.37 (1H, br d, *J*=14.2 Hz, H-21α), 4.08 (1H, d, *J*=14.2 Hz, H-21β), 3.83 (1H, d, *J*=4.6 Hz, H-15), 3.49 (1H, *J* partly overlapped with δ 3.46, H-5), 3.46 (1H, d, *J*=11.0 Hz, H-17), 3.26 (3H, s, N-Me), 3.23 (1H, *J* partly overlapped with δ 3.26, H-5), 3.04 (1H, br dt, *J*=15.0, 7.7 Hz, H-6), 2.85 (1H, d, *J*=11.0 Hz, H-17), 2.60 (1H, ddd, *J*=14.2, 10.7, 5.1 Hz, H-14β), 2.08 (1H, dd, *J*=15.0, 8.4 Hz, H-6), 1.76 (3H, dd, *J*=6.4, 1.5 Hz, H-18), 1.47 (1H, ddd, *J*=14.2, 5.4, 1.0 Hz, H-14α); ^{13}C NMR: Table 1.

Compound **9** (sweroside). Amorphous, UV λ_{max} nm: 241; FAB MS *m/z* : 359 $[M+H]^+$, ^{1}H NMR (500 MHz, CD_3OD) : δ 7.55 (1H, d, *J*=2.4 Hz, H-3), 5.52 (1H, ddd, *J*=18.5, 10.0, 8.6 Hz, H-8), 5.50 (1H, d, *J*=1.7 Hz, H-1), 5.26 (1H, dd, *J*=17.1, 1.8 Hz, H-10), 5.23 (1H, dd, *J*=10.2, 1.8 Hz, H-10), 4.63 (1H, d, *J*=7.8 Hz, H-1'), 4.41 (1H, ddd, *J*=11.1, 4.4, 2.2 Hz, H-7), 4.32 (1H, td, *J*=11.6, 2.6 Hz, H-7), 3.84 (1H, dd, *J*=12.0, 2.0 Hz, H-6'), 3.61 (1H, dd, *J*=12.0, 6.1 Hz, H-6'), 3.34-3.21 (3H, m, H-3', H-4', H-5'), 3.14 (1H, dd, *J*=9.3, 8.0 Hz, H-2'), 3.10 (1H, m, H-5), 2.65 (1H, ddd, *J*=9.8, 5.6, 1.7 Hz, H-9), 1.76-1.61 (2H, m, H-6); ^{13}C NMR (125.65 MHz, CD_3OD): δ 98.0 (C-1), 153.9 (C-3), 106.0 (C-4), 28.5 (C-5), 25.9 (C-6), 69.7 (C-7), 133.3 (C-8), 43.8 (C-9), 120.8 (C-10), 168.5 (C-11), glucosyl: 99.7 (C-1'), 74.7 (C-2'), 78.4 (C-3'), 71.5 (C-4'), 77.9 (C-5'), 62.7 (C-6').

INTRODUCTION

It is generally accepted that plants remain as an untapped reservior of potentially useful drugs, templates for synthetic modification and structure-activity studies. The selection of plants having a specified class of chemical compound by phytochemical screening, followed by designated bioassay models is one of scientific methods for the selection of plants that can be expected to contain novel biologically active compounds. Among naturally occurring compounds the indole alkaloids have provided many biologically active compounds. The majority of indole alkaloids have been isolated from the three plant families, Apocynaceae, Loganiaceae and Rubiaceae [1]. The genus *Alstonia* belongs to the tribe Plumerieae in the family Apocynaceae. This genus comprises of about 35 species which grow extensively in India, Southeast Asia, Polynesia and Australia. The plant *Alstonia glaucescens* (K. Sch.) Mona. is a tree measuring up to 20-25 m high. Pharmaceutical utilization, due to their alkaloid contents, of various species in this genus has been reported in many countries [2]. Phytochemical works of various species of the genus *Alstonia* especially in cases of *A. angustifolia* Miq., *A. macrophylla* Wall.ex G.Don and *A. scholaris* R.Br. are very interesting [3-5]. For the recent aspect, more than 130 indole alkaloids have been isolated from 25 different plants of this genus [6]. While the plant *Alstonia glaucescens* (K. Sch.) Mona. is still not chemically screened for alkaloids. The aim of this research project is to isolate and elucidate the structure of indole alkaloids from the stem bark of this particular plant.

RESULTS AND DISCUSSION

The crude alkaloids (16.5 g) were extracted by the usual procedure from the stem bark of *Alstonia glaucescens* (K.Sch.) Mona. The yield of alkaloid extract was 7.5 g / kg. Chemical constituents were separated by means of column chromatography (CC), medium pressure liquid chromatography (MPLC) and preparative thin-layer chromatography (prep. TLC). An intensive study resulted in the isolation of three new indole alkaloids (compounds **3**, **4** and **8**) along with one known iridoid (compound **9**) and five known indole alkaloids (compounds **1**, **2**, **5**, **6** and **7**). The proton and carbon assignments of the isolated compounds were mainly based on spectroscopic methods especially 1D- and 2D-NMR including ^{1}H-^{1}H COSY, PHSQC (^{13}C-^{1}H correlations), BCM, DEPT, differential NOE, and HMBC (long range ^{13}C-^{1}H correlations) experiments.

The UV and IR spectra of compounds **1**, **2** and **3** suggested the presence of β-anilinoacrylate chromophore. The mass spectra of **1** and **2** showed the same molecular peak at *m/z* 340 which agreed with a molecular formula $C_{20}H_{24}N_2O_3$. By comparison the details of ^{1}H and ^{13}C NMR data with the previous works [7, 8], compound **1** could be determined as echitamidine (C-20 *S*, C-19 *S*) and compound **2** as 20-epi-19ξ-echitamidine (C-20 *R*, C-19 ξ). The stereochemistry at C-20 in **2** was determined by differential NOE experiment. On irradiation of signal at δ 1.79 (H-20), the enhancements corresponding to H-6, H-21, H-15 and H-19 were observed which indicated the β-configuration of H-20 and led to the assignment of C-20 as 20 *R*.

The compound **3**, mp 187-188°C, showed UV and IR spectral data similar to those of **1**. The high resolution FAB mass spectrum of **3** showed a $[M+H]^+$ peak at *m/z* 357.1809, consistent with the molecular formula as $C_{20}H_{24}N_2O_4$. It is noteworthy that the molecular mass (EIMS) of **3** was 16 amu higher than that of **1**. The intensive fragmentation (23 %) of $[M-16]^+$ at *m/z* 340 is typical for N-oxide. In the ^{13}C NMR

spectrum (Table 1), C-3, C-5 and C-21 bound to N_b function, showed large downfield shifts while C-6 and C-20 upfield shifts in relation to **1**. This premise was further supported by ^1H NMR data which showed downfield shifts of H-3, H-5, H-20 and H-21 in relation to **1**. These data suggested the structure of **3** as echitamidine N-oxide which was confirmed by preparation of **3** from **1** by MCPBA oxidation.

Compound **9** exhibited a UV absorption at 241 nm which differed from those of common indole alkaloids. The mass spectrum (FAB) of **9** showed a peak at *m/z* 359 corresponding to $[M+H]^+$, whose molecular formula should be $C_{16}H_{22}O_9$. The ^1H and ^{13}C NMR data indicated the presence of one glucosyl group attached to the iridoid skeleton. By comparison the spectral data with the published paper [9], compound **9** could be assigned as sweroside.

The UV spectra of the five remaining compounds, **4**, **5**, **6**, **7** and **8** exhibited their close similarity of indoline chromophore. The main alkaloid, **5**, [$C_{21}H_{26}N_2O_4$, M_r 370], was determined to be N_b-demethylechitamine by comparison of physical and spectral data with previous work [10-12]. The high resolution FAB mass spectrum of **4** afforded a $[M+H]^+$ peak at *m/z* 413.2075 corresponding to the molecular formula $C_{23}H_{28}N_2O_5$. The molecular weight is 42 amu higher than that of **5**. The ^1H NMR signals of **4** at δ 4.79 (H-17), 3.73 (H-17) and 2.06 (Me), and ^{13}C NMR spectrum at δ 170.3 and 20.8 indicated the presence of C-17-O-acetyl moiety. While the chemical shifts and multiplicities of the remaining protons and carbons were similar to those of **5**. On acetylation with one equivalent of Ac_2O in pyridine, compound **5** gave the product which was identical with natural alkaloid (**4**). The structure of **4** was thus assigned to be 17-O-acetyl-N_b-demethylechitamine.

The mass spectrum (FAB) of **6** showed a peak at *m/z* 387 $[M+H]^+$, which represented a molecular formula $C_{21}H_{26}N_2O_5$ indicating 16 amu higher than that of **5**. By comparison spectral data of **6** with those of **5** and published work [10], compound **6** was concluded to be N_b-demethylechitamine N-oxide.

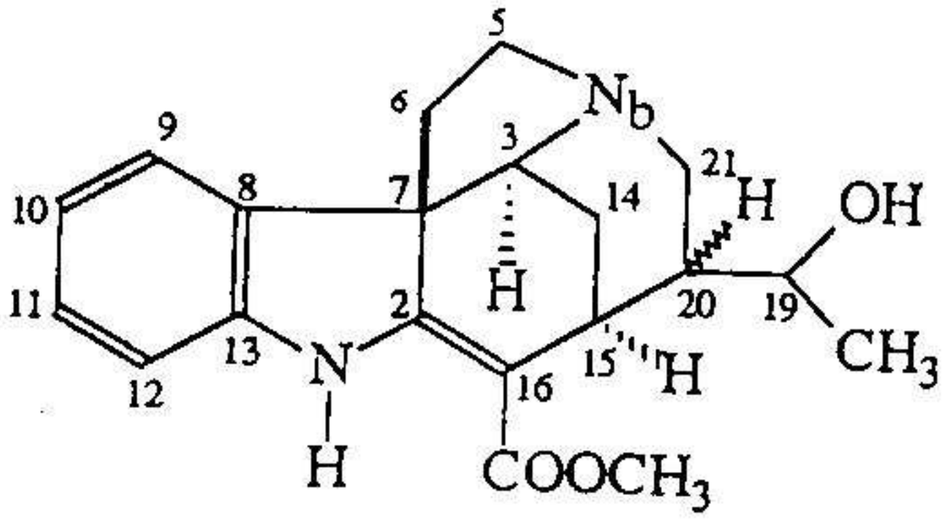
From the polar fraction (100 % MeOH) of the silica gel column (Fr. E), two quaternary alkaloids (7 and **8**) were isolated. By comparison with the published spectral data, alkaloid 7 [$C_{22}H_{29}N_2O_4$, M_r 385], was identified as echitamine [11-15]. The mass spectrum of 7 measured under EI condition was characterized by its thermal decomposition product at *m/z* 384 $[M-H]^+$, formed by a Hofmann degradation.

Alkaloid **8**, mp 218-220°C, showed pseudo-molecular ion peak at *m/z* 409 $[M+K]^+$ in the FAB mass spectrum, and afforded a $[M+H]^+$ peak from high resolution FAB mass spectrum at *m/z* 371.1967 establishing the molecular formula $C_{21}H_{26}N_2O_4$. This is 15 amu less than that of 7. A more detailed elucidation of the structure of **8** was obtained from ^1H and ^{13}C NMR spectra. The ^1H-^1H coupling informations obtained from ^1H-^1H COSY, one-bond correlations between proton and carbon nuclei gained from PHSQC and ^{13}C NMR (BCM and DEPT) spectral analyses figured the presence of two methyl, five methylene and seven methine functions. The other six remaining carbons were assigned as quaternary carbons including the C=O function resonated at δ 175.4. The cross peaks of ^{13}C-^1H long range correlations obtained from the HMBC experiments (Table 2) allowed the various fragments to be connected together. The numbers and multiplicities of the four aromatic protons of **8** at δ 7.70 (H-9), 6.99 (H-11), 6.68 (H-12) and 6.64 (H-10) suggested the lack of any substituent on the aromatic ring. The characteristic signals in ^1H and ^{13}C NMR spectra of the N_b-Me group were easily located at δ 3.26 (s) and δ 49.0, respectively. In order to elucidate the stereochemistry at C-16, C-3 and C-19 of **8,** differential NOE experiments were carried out. When the signals at δ 2.60 (H-14β) was irradiated, enhancements corresponding to H-3, H-14α, H-15 and H-17 were observed. Furthermore, no NOE enhancement of the two H-6 protons was observed upon the irradiations of H-17 protons. These led to the assignment of C-16 as C-16*R*. On irradiation of the signal at δ 4.45 (H-3), the enhancement of H-14β at δ 2.60 was observed. This result suggested the configuration of C-3 to be C-3*S*. Irradiation of the signal at δ 1.76 (H-18) gave enhancements of H-19 and H-15 which resulted in the assignment of C-19 as 19*E*. A notable point was the

absence of the methyl signal in the ester function. On the basis of the above data and comparison with those of **5** and **7** resulted in the assignment of alkaloid **8** to be echitaminic acid.

Chemical structures of the isolated compounds

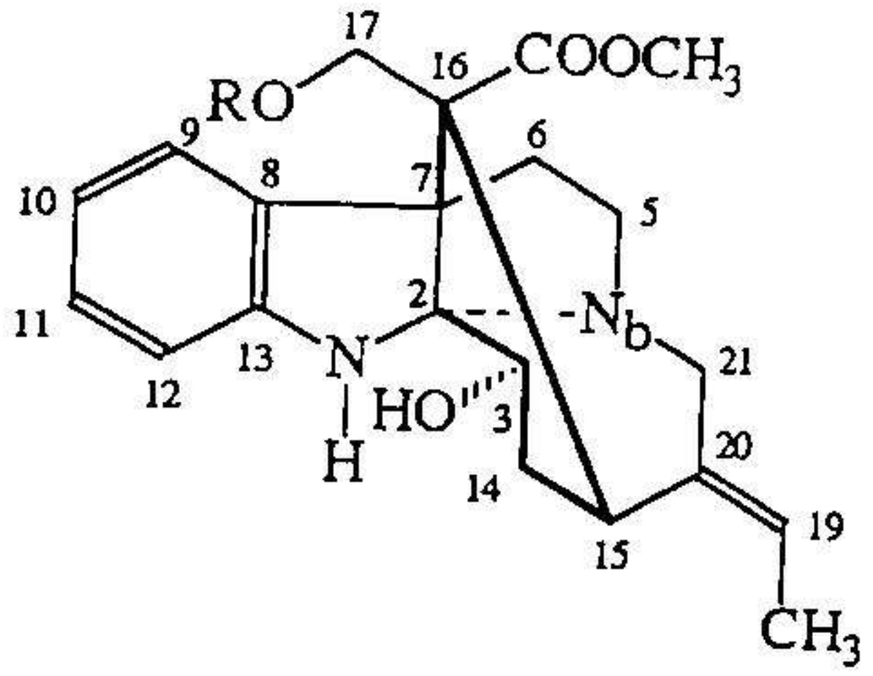
from the stem bark of *Alstonia glaucescens* (K. Sch.) Mona.



1 H-20α, C-20 *S*, C-19 *S*

2 H-20β, C-20 *R*, C-19 ξ

3 H-20α, C-20 *S*, C-19 *S*, $N_b \rightarrow O$

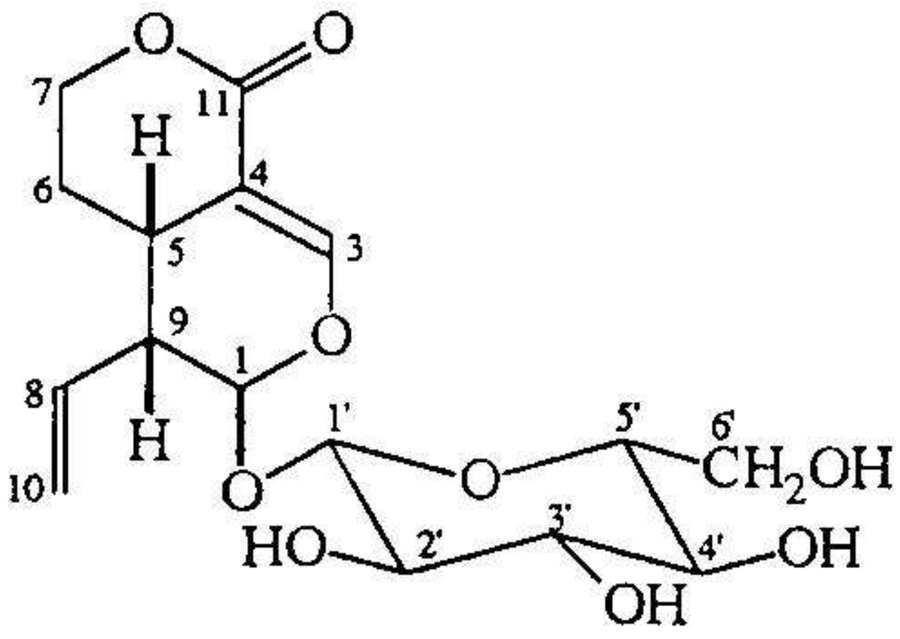


4 R = Ac

5 R = H

6 R = H, $N_b \rightarrow O$

7 R = H, N_b-CH_3



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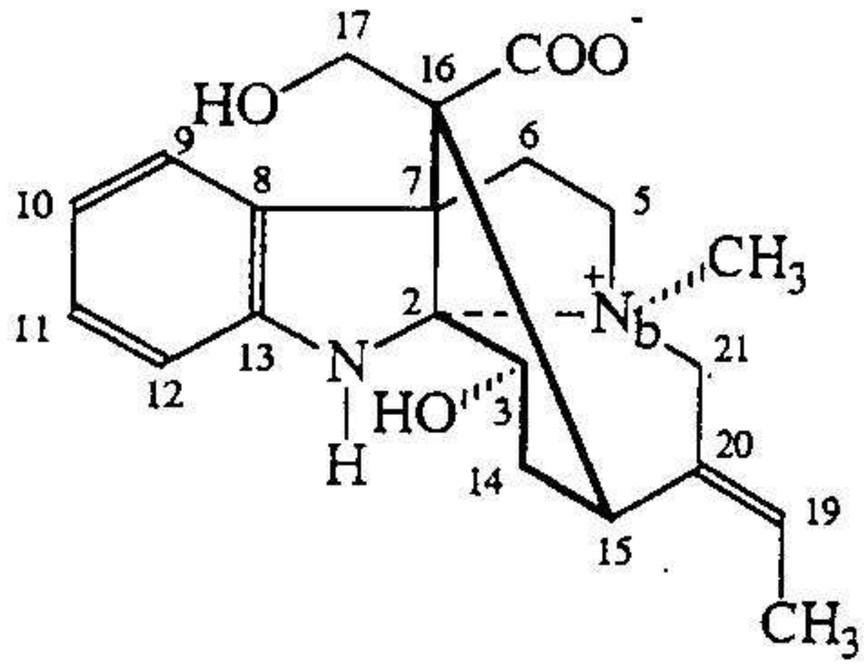

8

CONCLUSION AND RECOMMENDATION

The present work on the stem bark of *Alstonia glaucescens* (K. Sch.) Mona. has led to the isolation of eight indole alkaloids and one iridoid compound, sweroside. Three of the eight indole alkaloids, 17-O-acethyl-N_b-demethylechitamine, echitaminic acid and echitamidine N-oxide have never been reported from any sources and considered to be new indole alkaloids. The remaining five are known indole alkaloids namely, echitamidine, N_b-demethylechitamine, N_b-demethylechitamine N-oxide, echitamine and 20-epi-19ξ-echitamidine.

The presence of these indole alkaloids from this particular plant is an important supporting evidence for chemotaxonomy and biosynthetic study of chemical constituents from plants in the family Apocynaceae. The biological evaluation and chemical modification of the three new indole alkaloids remain of great intrinsic scientific interest for the further investigation.

Table 1. ^{13}C NMR assignments [δ (ppm), 125.65 MHz] of **1-8**

C	**1**[a]	**2**[a]	**3**[c]	**4**[a]	**5**[b]	**6**[b]	**7**[c]	**8**[c]
2	168.8	167.6	166.3	95.4	95.5	97.3	100.0	100.5
3	60.9	59.0	73.9	69.2	69.3	70.4	68.8	69.8
5	54.0	53.9	68.0	54.1	54.2	65.9	61.8	62.4
6	43.4	46.7	38.8	46.6	46.9	40.1	41.1	42.3
7	57.1	58.6	52.8	61.1	61.1	57.9	60.6	61.3
8	135.5	135.7	134.1	130.8	131.1	132.6	128.7	132.5
9	121.4	121.1	119.9	127.2	127.0	126.1	126.7	127.2
10	119.8	120.8	120.7	118.8	118.2	119.2	119.5	118.8
11	127.6	127.8	128.0	128.5	128.3	129.1	128.7	127.7
12	109.6	109.6	110.4	109.9	109.4	109.6	110.6	110.0
13	147.7	143.7	144.1	148.6	148.9	147.2	147.5	147.2
14	31.0	27.3	27.2	32.5	32.0	31.9	30.7	31.9
15	28.8	29.3	27.6	36.0	35.9	36.1	34.4	37.0
16	96.9	102.7	98.1	54.3	56.3	55.3	55.7	53.1
17	-	-	-	67.1	66.7	66.0	64.5	65.9
18 (Me)	19.8	20.2	20.1	14.8	14.5	14.4	14.9	14.1
19	68.4	71.1	66.4	123.6	122.9	127.9	129.8	127.3
20	45.8	45.5	41.4	138.5	139.4	129.9	132.6	135.1
21	48.1	48.3	62.6	57.4	57.3	74.1	64.7	65.3
COOMe	172.3	168.8	167.7	173.3	175.1	173.5	173.1	175.4
COOMe	51.9	51.4	51.4	51.9	51.9	52.0	51.9	-
OAc	-	-	-	170.3	-	-	-	-
OAc	-	-	-	20.8	-	-	-	-
N_b-Me	-	-	-	-	-	-	49.6	49.0

[a] In $CDCl_3$, [b] In $CDCl_3$-CD_3OD, [c] In DMSO-d_6

Table 2. Long range ^{13}C-^{1}H correlations observed in the HMBC of **8**

δ ^{1}H	δ ^{2}J CH	δ ^{3}J CH
7.70 (H-9)		127.7 (C-11), 147.2 (C-13)
7.30 (NH)		61.3 (C-7), 132.5 (C-8)
6.99 (H-11)		127.2 (C-9), 147.2 (C-13)
6.68 (H-12)		118.8 (C-10), 132.5 (C-8)
6.64 (H-10)		110.0 (C-12), 132.5 (C-8)
5.66 (H-19)	14.1 (C-18)	37.0 (C-15), 65.3 (C-21)
4.08 (H-21β)	135.1 (C-20)	37.0 (C-15), 100.5 (C-2), 127.3 (C-19)
3.83 (H-15)	31.9 (C-14)	61.3 (C-7), 65.3 (C-21),
	53.1 (C-16)	69.8 (C-3), 127.3 (C-19)
	135.1 (C-20)	
3.49 (H-5)		61.3 (C-7)
3.46 (H-17)		37.0 (C-15), 61.3 (C-7), 175.4 (C=O)
3.26 (N-Me)		62.4 (C-5), 65.3 (C-21), 100.5 (C-2)
3.23 (H-5)		61.3 (C-7), 65.3 (C-21)
3.04 (H-6)	61.3 (C-7)	53.1 (C-16), 132.5 (C-8)
2.85 (H-17)	53.1 (C-16)	175.4 (C=O)
2.08 (H-6)	61.3 (C-7)	100.5 (C-2), 132.5 (C-8)
1.76 (H-18)	127.3 (C-19)	135.1 (C-20)
1.47 (H-14α)	37.0 (C-15)	53.1 (C-16), 135.1 (C-20)
	69.8 (C-3)	

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