Phytochemistry, Vol. 29, No. 3, pp. 969–972, 1990.
Printed in Great Britain.

0031-9422/90 $3.00 + 0.00

STEROIDAL ALKALOIDS FROM *HOLARRHENA ANTIDYSENTERICA**

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(*Received* 20 *February* 1989)

Key Word Index—*Holarrhena antidysenterica*; Apocynaceae; bark; steroidal alkaloids; regholarrhenine D; regholarrhenine E; regholarrhenine F.

Abstract—Three new steroidal alkaloids, regholarrhenine D, E and F, along with the known alkaloid kurchessine, were isolated from the stem bark of *Holarrhena antidysenterica*. The structures were elucidated on the basis of spectral and chemical evidence. It was shown that the first alkaloid possesses the *endo* N–OH function in lieu of the *endo* N–Me function in conessine. The second alkaloid is the C-7 stereoisomer of the previously isolated alkaloid kurcholessine. The third alkaloid was established as the 18-hydroxymethyl analogue of kurchessine.

INTRODUCTION

In our earlier communication [2], we reported the isolation and characterization of three steroidal alkaloids, regholarrhenine A–C, from the stem bark of *Holarrhena antidysenterica* collected from the wild locations of the sub-Himalayan region. We now describe the isolation and characterization of three new alkaloids, in addition to conessine [3] and kurcholessine [4], from the stem bark of *H. antidysenterica* collected at flowering stage from the western temperate region of India. These alkaloids have been named regholarrhenine D, E and F. Some scanty information about the tentative structure of regholarrhenine F is available in the literature where the same compound is named as tetramethylholarrhemine [5, 6].

RESULTS AND DISCUSSION

The total alkaloidal fraction (0.85% yield) isolated from the stem bark of the plant was chromatographed on basic alumina using gradient elutions of petrol, ethyl acetate and methanol. Initial fractions from petrol yielded conessine (0.11%) and further elution with petrol–ethyl acetate (9:1) gave a mixture (0.15%) of partially demethylated analogues of conessine, viz. conessimine [7] and isoconessimine [8]. Regholarrhenine D (**1**; 0.001% yield), kurcholessine (**5**; 20 mg) and regholarrhenine E (**6**; 0.001% yield) were obtained from the petrol–ethyl acetate (4:1), ethyl acetate and ethyl acetate–methanol (99:1) eluates respectively. Further elution of the column with methanol afforded regholarrhenine F (**8**; 0.4% yield).

Regholarrhenine D (**1**), mp 255–257°, $[M-17]^+$ at *m/z* 341 ($C_{23}H_{38}N_2O$) had properties characteristic of a conenine derivative and closely resembled conessine (**4**) [3]. Its IR spectrum showed absorptions at 3460 (OH), 1375 [$N(Me)_2$], 1040 (OH bending) and 980 (N–O st.). In the mass spectrum, the appearance of the base peak at *m/z* 84, another prominent peak at *m/z* 57, an $[M-17]^+$ peak at *m/z* 341 and a small peak at *m/z* 326 indicated the presence of the *N*-hydroxy group in ring E in the light of the known fragmentation pattern of con-5-enine derivatives [9]. In the ^1H NMR spectrum of **1**, the differences from the spectrum of conessine (**4**) were the presence of a D_2O exchangeable proton signal at δ4.80, the deshielded C-21 Me proton signal at δ1.34 and the absence of an endo N-Me proton signal. However, on D_2O exchange the C-21 proton signal was shielded to δ1.09. Thus, the hydroxyl function was placed on the 18,20-epimino group. The only other position where the hydroxyl group could be placed was at an exo nitrogen. However, this position was discounted on the basis of the mass spectrum of **1**.

Acetylation of base **1** gave a sticky *N*-acetoxy product (**2**), which failed to crystallize even after passing through a column; ν_{max}^{KBr} 1750 (C=O of N–OAc). Its ^1H NMR spectrum showed the presence of a singlet at δ2.13 which has been assigned to an *N*-acetoxy group. As expected, the C-21 proton signal was deshielded by 0.23 ppm from the corresponding signal in **4**.

Zinc–acetic acid reduction of **1** gave **3** which was converted into **4** with a mixture of formic acid and formaldehyde. It is well established that the reduction of hydroxylamine normally results in cleavage of the N–O bond to produce amines [10]. The *N*-hydroxylamine structure (**1**) is thus conclusively established. However, **1** was also converted into **4** with a mixture of formic acid and formaldehyde evidently by the *N*-methylation reaction of the conessimine generated *in situ* due to the presence of reducing agent formic acid. The physical and spectroscopic data for kurcholessine (**5**) agreed with the literature data [4].

Regholarrhenine E (**6**), mp 285–287°, $[M]^+$ at *m/z* 404 ($C_{25}H_{44}N_2O_2$) had properties similar to **5**. Its IR absorptions at 3520 (OH), 3150 (OH), 1200 (OH bending) and

* Part 12 in the series 'Investigations of Medicinal Plants'. For Part 11 see ref. [1].

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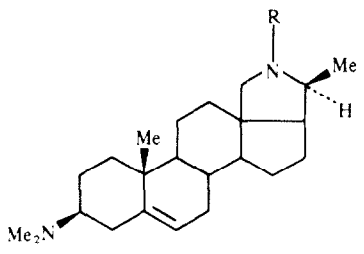
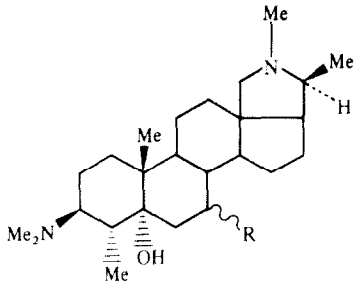
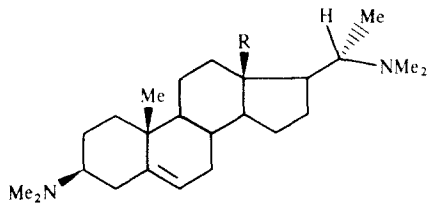


1 R = OH
2 R = OAc
3 R = H
4 R = Me



5 R = β-OH
6 R = α-OH
7 R = O



8 R = CH_2OH
9 R = CHO
10 R = Me

1160 (OH bending) cm^{-1} and fingerprint region had similarities with **5** suggesting **6** to be isomeric with **5**. The molecular ion peaks and fragmentation patterns of both **5** and **6** were similar. In the mass spectrum, the appearance of strong $[M-15]^+$ and $[M-17]^+$ peaks, along with prominent peak at *m/z* 372 originating by the loss of 17 mass units from the $[M-15]^+$ peak, further confirmed the isomeric nature of **5** and **6**. The dichotomy between structures **5** and **6** was resolved by comparison of the ^1H NMR spectra.

The spectra were almost identical except for the chemical shifts and contours of one proton signal. A narrow band at δ3.52 (*br s*, 1H, $W_{1/2}$ 5.5 Hz) in **6** and a broad band at δ3.10 (*br s*, 1H, $W_{1/2}$ 22 Hz) in **5** suggested that the isomerism occurred at C-7. The position of the hydroxyl function was axial and equatorial in **6** and **5**, respectively. The C-25 proton and axial proton (at C-4; J = 10 Hz) signals were found at δ1.29 and 2.90, respectively. Both **5** and **6** gave on Jones oxidation the same oxo derivative which conclusively established the hydroxyl position at C-7 in **6**.

Regholarrhenine F (**8**) mp 210–212°, $[M]^+$ at *m/z* 388 ($C_{25}H_{44}N_2O$) had properties characteristic of a 3,20-diamino pregnene [11–13] and resembled closely the 18-hydroxy derivative [12]. Its IR spectrum showed the presence of a hydroxy function with absorptions at 3400 and 1030 (bending) cm^{-1}. In the mass spectrum, the appearance of a base peak at *m/z* 72 and another prominent peak at *m/z* 84 were in agreement with a 3,20-diaminopregnene structure (**8**) for regholarrhenine F. Moreover, the loss of these two fragments from the whole structure was evident by the presence of fragment ions at *m/z* 316 $[M-72]^+$ and *m/z* 288 $[M-15-84]^+$. The $[M]^+$, $[M-15]^+$ and $[M-31]^+$ peaks were of low relative intensity.

In the ^1H NMR spectrum of **8**, the singlet at δ3.46 integrating for two protons was assigned to the C-13 hydroxymethylene protons due to the absence of any other proton–proton interactions. The only other position where this hydroxymethylene group could be placed was at C-10 but this was excluded on the basis of no unusual chemical shift of the C-6 protons [7]. The vinylic proton was found at δ5.38 as a broad singlet. The two proton wide multiplet ($W_{1/2}$ 22 Hz) at δ2.92 and two singlets for six protons each at δ2.32 and δ2.30 were assigned to the C-3α-, C-20β-, C-3β-NMe_2 and C-20α-NMe_2, respectively. The C-21 (*d*, 3H, J = 12 Hz) and C-19 (*s*, 3H) methyl groups appeared at δ1.08 and 1.06 with a D_2O-exchangeable singlet at δ2.16.

The above data confirmed the tentative structure of tetramethylholarrhimine reported in the literature [5, 6]. However, there was a wide difference in the melting points of the two compounds [regholarrhenine F mp 210–212°; tetramethylholarrhimine mp 227–228°] and our efforts to enhance the mp of **8** by repeated crystallizations did not succeed.

Jones oxidation of **8** gave an oxo derivative **9**, mp 125–127°; ν_{max}^{KBr} cm^{-1}: 2750 (CHO st.) and 1705 (C=O). Its mass spectrum showed peaks at *m/z* 371 and 357 which resulted by the loss of 15 (Me) and 29 (CHO) mass units from the weak molecular ion peak at *m/z* 386. The base peak at *m/z* 84 and another almost equally intense (95%) peak at *m/z* 72 originated from the cleavage of the C-3/C-4 bond in the 3-amino-pregnene system and from the cleavage of the C-17/C-20 bond from the rest of the steroidal molecule with the formation of $CH_2=CH-CH=\overset{+}{N}-Me_2$ and $CH_3-CH=\overset{+}{N}-Me_2$ species, respectively. In the ^1H NMR spectrum of **9**, the proton singlet at δ0.88 (3H) and the doublet (3H, J = 8 Hz) at δ0.95 were assigned to the C-19 and C-21

Table 1. ^{13}C NMR chemical shifts of compounds **1**, **6** and **8** in $CDCl_3$

	Carbon							
	1	2	3	4	5	6	7	8
1	38.3	24.9	64.8	35.3	141.8	120.3	31.8	33.7
6	38.5	26.5	63.8	34.0	74.5	26.5	75.0	33.0
8	38.5	25.0	65.0	35.2	141.0	120.0	32.8	38.5
	9	10	11	12	13	14	15	16
1	49.8	37.8	21.8	26.7	50.5	55.3	22.5	36.5
6	58.5	37.5	21.5	26.5	50.6	54.5	22.2	44.5
8	51.0	37.0	20.5	32.0	47.0	56.2	23.8	35.2
	17	18	19	20	21	22	23	24
1	53.1	64.8	19.3	56.5	14.8	41.6	41.6	—
6	53.5	63.8	16.0	63.0	13.5	40.0	40.0	40.8
8	55.5	59.0	19.5	59.0	11.5	41.6	41.6	42.0
	25							
1	—							
6	23.5							
8	42.0							

protons, respectively. The aldehydic proton, however, was not traceable. The signals at δ5.32 (*br s*, 1H), 2.50 (*d*, 2H, $J = 14$ Hz), 2.28 (*s*, 6H) and 2.15 (*s*, 6H) were assigned to C-6, C-3α and C-20β, $N(Me)_2$ and $N(Me)_2$ protons, respectively. The most unequivocal proof for the structure **8** for regholarrhenine F came from the Wolff–Kishner reduction of the oxo compound (**9**) which afforded the known alkaloid kurchessine (**10**) [14].

Finally, ^{13}C NMR (Table 1) values for **1**, **6** and **8** have been obtained which conclusively established their assigned structures. The signal assignments for the carbons were deduced by comparison with those of conessine [15] and other pregnene derivatives [16].

EXPERIMENTAL

Mps: uncorr. ^1H NMR spectra were recorded at 60 MHz. ^{13}C FT NMR at 22.49 MHz in $CDCl_3$ with TMS as an int. std in 5 mm spinning tubes at room temp. Concns of compounds were *ca* 0.6–0.8 mmol/ml. FT-measurement conditions were: spectral width 5648 Hz; pulse flipping angle *ca* 45; acquisition time 0.75 sec; numbers of data points 5000. CC was carried out using basic Al_2O_3. TLC was run on silica gel in C_6H_6–EtOAc–Et_2NH (6:3:1) and spots were developed by Dragendorff's reagent.

Plant material. Stem bark of *H. antidysenterica* (L.) Wall was collected from the wild locations of the western temperate region.

Isolation of alkaloids. Air-dried and powdered stem bark (10 kg) was extracted exhaustively with 2 M HCl over a period of 4 days. The 2M HCl soluble portion was extracted with $CHCl_3$ (5 × 10 l) to remove neutral components. The aq. acidic layer was then made alkaline (pH 8.5) with NH_4OH soln (30%) and repeatedly extracted with $CHCl_3$ (5 × 10 l). The combined extracts were washed with H_2O, dried and evapd *in vacuo* to yield crude total alkaloids (85 g, 0.85%) as a dark brown sticky mass. On TLC this showed three major and one minor spot and some polar portion at the base. The crude extract was subjected to CC (2 kg) after the formation of a slurry. Fractions 1–7 eluted with petrol (60–80°) gave conessine (**4**, 11 g, 0.11%) after crystallization from Me_2CO.

Fractions 8–15 from CC eluted with 10% EtOAc in petrol (60–80°) gave a mixture of conessimine and isoconessimine (15 g; 0.15%) as shown by comparative TLC with authentic samples. On methylation it was converted into **4**.

Fractions 16–20 from CC eluted with 20% EtOAc in petrol afforded regholarrhemine D (**1**) as a white amorphous solid from EtOAc (0.152 g; 0.001%), mp 255–257°, IR ν_{max}^{KBr} cm^{-1}: 3460, 3180, 2940, 2795, 1650, 1460, 1375, 1040 and 980. ^1H NMR: δ5.36 (*s*, 1H, C-6), 4.80 (*s*, 1H, D_2O exchangeable N-OH), 2.90 (*d*, 2H, $J = 8$ Hz, C-3α and C-20αH), 2.67 (*m*, 2H, C-18), 2.34 (*s*, 6H, NMe_2), 1.34 (*d*, 3H, $J = 7$ Hz, C-21, on D_2O addition doublet shifts to δ1.09) and 0.96 (*s*, 3H, C-19); MS *m/z* (rel. int.): 341 $[M-17]^+$ (60), 326 (18), 85 (99.8) (due to zwitterion behaviour), 84 (100), 57 (95) and 28 (60). ($C_{23}H_{38}N_2O$ requires C, 77.09; H, 10.61; N, 7.82. Found C, 77.40; H, 10.66 and N, 7.67%). Acetylation of **1** with Ac_2O–pyridine at room temp gave a sticky acetoxy product (**2**) after purification over a column. IR ν_{max}^{KBr} cm^{-1}: 2940, 1750, 1430, 1330, 1200, 1040 and 980. ^1H NMR δ5.29 (*br s*, 1H, C-6), 3.16 (*m*, 2H, C-18), 3.03 (*d*, 1H, $J = 10$ Hz, C-3α H), 2.70 (*d*, 1H, $J = 8$ Hz, C-20αH), 2.30 (*s*, 6H, NMe_2), 2.13 (*s*, 3H, N-OAc), 1.29 (*d*, 3H, $J = 6$ Hz, C-21) and 0.97 (*s*, 3H, C-19). Zn–HOAc redn of **1** gave **3**, TLC and mmp comparable. Methylation of **1** with HCHO-HCO_2H gave **4** (comparable mp, mmp, superimposable IR, ORD and CD curves).

CC fractions 27–28 eluted with EtOAc afforded a known alkaloid kurcholessine (**5**, 0.02 g), mp 214–216° (218–221° lit.) which was confirmed by the Jones oxidation of kurcholessine to the oxo-compd **7**.

CC fractions 30–36 eluted with 1% MeOH in EtOAc afforded regholarrhenine E(**6**) as a brown solid from MeOH (0.155 g; 0.0015%), mp (285–287°). IR ν_{max}^{KBr} cm^{-1}: 3520, 3150, 2900, 1450,

1420, 1360, 1200, 1160 and 1010. ^{1}H NMR: δ4.45 (*s*, 1H, D_2O exchangeable), 3.52 (*br s*, 1H, $W_{1/2}$ 5.5 Hz, C-7βH), 3.45 (*br s*, 1H, D_2O exchangeable), 3.07 (*dd*, 2H, $J=12$, 4 Hz, C-3α and C-20αH), 2.90 (*d*, 1H, $J=10$ Hz, 4β-H axial), 2.32 (*s*, 6H, NMe_2), 2.25 (*s*, 3H, NMe), 1.29 (*d*, 2H, $J=10$ Hz, C-25), 1.09 (*s*, 3H, C-19) and 1.03 (*d*, 3H, $J=6$ Hz, C-21). MS *m/z* (rel. int.): 404 $[M]^+$, (<0.1%), 389 $[M-15]^+$ (5), 387 $[M-17]^+$ (38), 374 (70), 372 (22), 264 (18), 253 (35), 252 (25), 209 (65), 142 (45), 108 (25), 98 (65), 84 (90), 71 (99), 32 (100) and 28 (98). ($C_{25}H_{44}N_2O_2$ requires C, 74.25; H, 10.89; N, 6.93%). Found C, 72.90; H, 10.38, and N, 6.60%).

Jones oxidation of **6** and usual work-up gave an oxo compound (**7**), from Me_2CO mp 210–212°. IR ν_{max}^{KBr} cm^{-1}: 3450, 2940, 1710, 1600 and 1450. ORD (*c*, 0.2): $[\Phi]_{400}-620°$, $[\Phi]_{350}-640°$, $[\Phi]_{335}-690°$, $[\Phi]_{324}-830°$, $[\Phi]_{312}-700°$, $[\Phi]_{250}-250°$, $[\Phi]_{223}0°$, $[\Phi]_{215}+80°$, $[\Phi]_{211}0°$. CD $[\Theta]_{350}$ $-90°$, $[\Theta]_{325}-450°$, $[\Theta]_{308}-1130°$, $[\Theta]_{295}-900°$, $[\Theta]_{280}$ $-400°$, $[\Theta]_{250}-120°$.

CC fractions 40–45 eluted with pure MeOH yielded after methylation with HCHO–HCOOH a white crystalline solid from MeOH (4 g; 0.04%) identified as regholarrhenine F (**8**), mp 210–212° (lit. 227–228°). IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1460 and 1030. ^{1}H NMR: δ5.38 (*br s*, 1H C-6H), 3.46 (*s*, 2H, C-18), 2.92 (*m*, 2H, $W_{1/2}$ 22 Hz, C-3α and C-20β H) 2.32 (*s*, 6H, NMe_2), 2.30 (*s*, 6H, NMe_2), 2.16 (*s*, 1H, D_2O-exch.), 1.08 (*d*, 3H, $J=12$ Hz, C-21) and 1.06 (*s*, 3H, C-19), MS *m/z* (rel. int.): 388 $[M]^+$ (<1%), 373 (1), 342 (1), 316 (2.34), 289 (2), 84 (63), 72 (100), 58 (10.7), 44 (10), 28 (90). ($C_{25}H_{44}N_2O$ requires C, 77.06; H, 11.34; N, 7.22. Found C, 76.88; H, 11.02; N, 7.37%).

CrO_3–HOAc oxidation of **8** at room temp. for 24 hr afforded the oxo-compound as white needles from MeOH, mp 125–127°, IR ν_{max}^{KBr} cm^{-1}: 2920, 2910, 2750, 1705, 1460, and 1165. ^{1}H NMR: δ5.32 (*br s*, 1H, C-6), 2.50 (*d*, 2H, $J=14$ Hz, C-3αH and C-20βH), 2.28 (*s*, 6H, NMe_2), 2.15 (*s*, 6H, NMe_2), 0.95 (*d*, 3H, $J=8$ Hz, C-21) and 0.88 (*s*, 3H, C-19). MS *m/z* (rel. int.): 386 $[M]^+$ (<1%), 371 (<1), 319 (1.3), 287 (3), 84 (100), 72 (95), 40 (60), 32 (95) and 29 (65).

Wolff–Kishner reduction of the oxo-compd (**9**) afforded the known alkaloid kurchessine (**10**), mp 137–138° (lit. mp 140–142°, identical mp, mmp and superimposable IR).

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