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C_{21} steroidal glycosides from *Hemidesmus indicus*

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Abstract

Two novel pregnane glycosides, denicunine (**1**) and heminine (**4**), have been isolated from the dried stem of *Hemidesmus indicus* R.Br. (family: Asclepiadaceae). Chemical transformations and spectroscopic evidence viz: ^{1}H and ^{13}C NMR spectroscopy and FABMS are consistent with the structures calogenin 3-*O*-3-*O*-methyl-β-D-fucopyranosyl-(1 → 4)-*O*-β-D-oleandropyranoside and calogenin 3-*O*-β-D-cymaropyranosyl-(1 → 4)-*O*-β-D-digitoxopyranoside, respectively.

Keywords: *Hemidesmus indicus*; Asclepiadaceae; Pregnane glycosides; Denicunine; Heminine; Calogenin; C_{21} steroids

1. Introduction

India, with its varied climatic conditions, is a repository of a rich and diverse flora, which is the mainstay of well organised Indian traditional systems of medicine viz. Ayurveda and Siddha. Plants belonging to family Asclepiadaceae which have been reported to be rich in pregnane and cardiac glycosides (Deepak et al., 1996, 1997a, 1997b) are widely distributed throughout India and frequently used in traditional medicines. Studies have shown that pregnanes and their glycosides possess antitumour and anticancer activities (Itokawa et al., 1988; Umehara et al., 1994; Deepak et al., 1996, 1997a, 1997b). *Hemidesmus indicus* R.Br. (common name: Anantmul) (synonyms: *H. wallichii*, *Periploca indica*, *Asclepias pseudosarsa*) is a medicinal, perennial, prostrate and twining shrub and is being used against syphilis, leucorrhoea, bronchitis, chronic rheumatism, urinary diseases, leprosy, leucoderma and skin diseases, and as purgative, diaphoretic, diuretic, antipyretic and antidiarrhoeal in folk medicines (Kirtikar and Basu, 1984). Earlier we have reported the isolation of nine pregnane glycosides viz. Desinine (Oberai et al., 1985), Indicine, Hemidine (Prakash et al., 1991), Indicusin (Deepak et al., 1995), Hemidescine, Emidine (Chandra et al., 1994), Medidesmine, Hemisine and Demicine (Deepak et al., 1997b) from *H. indicus*. We now report the structure of two novel pregnane oligoglycosides from $CHCl_3$–EtOH (3:2)-soluble extract of dried stems of *H. indicus*.

2. Results and discussion

Denicunine (**1**) {mp 148°C, $[\alpha]_D + 20°$, $C_{35}H_{58}O_{10}$ FABMS (*m*/*z* 677 $[M + K]^+$)} responded positively to the Liebermann–Burchardt (Abisch and Reichstein, 1960), Xanthydrol (Khare et al., 1984), Keller–Killiani (Khare et al., 1984) and Feigl tests (Feigl, 1975), indicating it to be a steroidal glycoside of 2,6-dideoxy and normal hexose(s). The presence of two anomeric protons and carbons at δ 4.62, 4.30 and δ 102.9, 102.2 in its ^{1}H and ^{13}C NMR spectra, respectively, suggested it to be a diglycoside. The ^{1}H NMR spectrum also exhibited the presence of one 2,6-dideoxy and one 6-deoxy sugar in **1**.

The aglycone and the sugars were identified as calogenin (Srivastava et al., 1982), D-oleandrose (Renkonen et al., 1959) and 3-*O*-methyl-D-fucose (Collins,

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1987) by comparing the hydrolysate mixture with authentic samples and converting them into known derivatives (Renkonen et al., 1959; Collins, 1987). The aglycone and monosaccharides of **1** were identified by Mannich and Siewert hydrolysis (Mannich and Siewert, 1942) (with TLC and PC monitoring). After 2 days, the hydrolysate showed two new spots besides the starting material. The less polar spot was identical in mobility with the authentic sample of calogenin (Srivastava et al., 1982) (PC, TLC), while the polar spot was due to the glycon, presumably a disaccharide. Hydrolysis was complete in 4 days which gave two new spots with the disappearance of the polar spot. These new spots were identified as D-oleandrose and 3-*O*-methyl-D-fucose by comparison with the authentic samples (TLC, PC). The presence of disaccharide by the hydrolysis suggested that the sequence in which the sugars are attached to **2** was D-oleandrose and 3-*O*-methyl-D-fucose.

The large coupling constants of the anomeric protons of D-oleandrose and 3-*O*-methyl-D-fucose present as double doublet at 4.62 (J = 8 Hz and 2 Hz) and doublet at δ 4.30 (J = 8 Hz), respectively, in the ^{1}H NMR spectrum of **1** were typical of their axial configuration in hexopyranoses in $^4C_1$ (D) conformation, having β-glycosidic linkage (Allgeier, 1968). The ^{1}H NMR spectrum also contained other relevant proton signals of the diglycoside (see Table 1). The data of ^{13}C NMR spectrum, which was in close conformity with the ^{1}H NMR data of **1**, further confirmed the deduced structure. A relatively downfield appearance of the two anomeric carbons at δ 102.9 and 102.2 supplemented β-glycosidic linkages for two sugar units. It also contained other significant signals of sugar and genin moieties in the spectrum. The chemical shifts of ^{13}C NMR signals are given in Table 2.

The position of glycosidic linkage of aglycone in **1** was confirmed by acetylation of **1** which afforded tri-*O*-acetyl denicunine (**3**) confirmed by the presence of three singlets of three protons each at δ 2.05, 2.03 and 1.99 in the ^{1}H NMR spectrum of **3**. The downfield shift of H-20 methine proton at δ 4.96–4.88 (Qui et al., 1997) in **3** suggested that the sugar was linked glycosidically to C-3 hydroxyl group of the aglycone.

The FAB mass of **1** showed the highest pseudo molecular mass ion peak at m/z 677 $[M + K]^+$ and a molecular ion peak at m/z 638 $[M]^+$ which was in conformity with the molecular formula $C_{35}H_{58}O_{10}$. The FAB mass not only supported the derived structure, but also confirmed the sequence and position of sugar–sugar–genin linkage in **1**. For convenience the sugars were designated as S_2 and S_1 starting from the terminal end. The mass ion peak at m/z 593 was attributed to the loss of the side chain at C-17 $[M - 45]^+$,

Table 1
^{1}H NMR spectral data for compounds **1** and **4**[a]

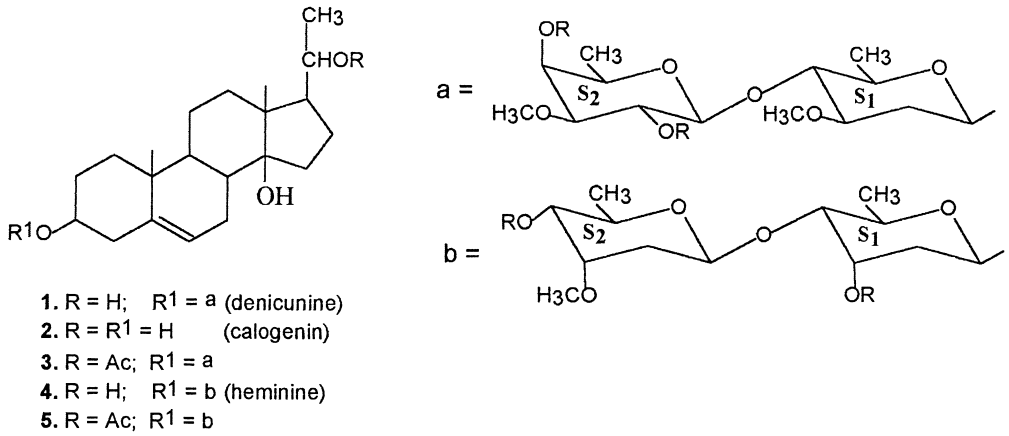
H (proton)	Compound **1**[b]	H (proton)	Compound **4**[c]
1′	4.62 *dd* (8 Hz, 2 Hz) S_1 (Ole)	1′	4.40 *dd* (8 Hz, 2 Hz) S_1 (Dig)
1″	4.30 *d* (8 Hz) S_2 (Fuc)	1″	4.54 *dd* (9 Hz, 2 Hz) S_2
2′ eq	2.24–2.16 *m* S_1	2′ eq	2.34–2.24 *m* S_1
2′ ax	1.70–1.58 *m* S_2	2″ eq	2.34–2.24 *m* S_2 (Cym)
2″	3.30 *t* (8 Hz) S_2	2′ ax	1.64–1.54 *m* S_1
3′	3.62–3.56 *m* S_1	2″ ax	1.64–1.54 *m* S_2
3″	3.62–3.56 *m* S_2	3′	3.86–3.84 *m* S_1
4′	3.12 *t* (9 Hz) S_1	3″	3.86–3.84 *m* S_2
4″	3.48–3.42 *m* S_2	4′	3.44–3.40 *m* S_1
5′	3.88–3.84 *m* S_1	4″	3.38–3.34 *m* S_2
5″	3.88–3.84 *m* S_2	5′	3.56–3.54 *m* S_1
6	5.38 *m*	5″	3.56–3.54 *m* S_2
6′ Me	1.35 *d* (6 Hz) S_1	6	5.36 *m*
6″ Me	1.32 *d* (6 Hz) S_2	6′ Me	1.34 *d* (6 Hz) S_1
21 Me	1.29 *d* (6 Hz)	6″ Me	1.32 *d* (6 Hz) S_2
18 Me	1.091 *s*	21 Me	1.26 *d* (6 Hz)
19 Me	0.74 *s*	18 Me	1.00 *s*
20	3.78–3.74 *m*	19 Me	0.88 *s*
OMe	3.52 *s* S_1	20	4.12–4.08 *m*
OMe	3.40 *s* S_2	OMe	3.52 *s* S_1

[a] Fuc = 3-*O*-methyl-D-fucose; Cym = D-cymarose; Ole = D-oleandrose; Dig = D-digitoxose.
[b] S_1 = D-oleandrose; S_2 = 3-*O*-methyl-D-fucose in compound **1**.
[c] S_1 = D-digitoxose; S_2 = D-cymarose in compound **4**.

Table 2
^{13}C NMR spectral data for compounds **1** and **4**[a]

C	Aglycone			Sugars		
	1	**4**	C	**1**	C	**4**
1	35.9[b]	35.2	1	102.9	1	101.0
2	27.9[c]	29.3[b]	2	74.3[b]	2	39.3[b]
3	79.3	78.1	3	75.0	3	72.2[c]
4	39.6	40.7	4	74.1[b]	4	74.4
5	139.0	140.8	5	69.6[c]	5	69.9
6	122.5	124.1	6	20.7	6	18.1
7	27.9[c]	29.3[b]	–OMe	61.2	–OMe	55.6
8	30.4[d]	32.2[c]	1	102.2	1	99.6
9	48.2	49.3	2	37.5	2	39.8[b]
10	37.1	38.5	3	78.3	3	71.5[c]
11	24.8	23.3	4	83.2	4	83.7
12	36.1[b]	37.0	5	70.0[c]	5	67.4
13	44.3	47.0	6	19.3	6	17.8
14	86.3	85.2	–OMe	57.1		
15	30.0[d]	31.5[c]				
16	27.7[c]	27.8				
17	55.0	50.7				
18	15.4	14.8				
19	17.9	16.3				
20	66.1	65.2				
21	22.5	21.1				

[a] Value (ppm) from internal TMS in $CDCl_3$.
[b–d] The assignments in each column may be interchangeable.



1. R = H; R^1 = a (denicunine)
2. R = R^1 = H (calogenin)
3. R = Ac; R^1 = a
4. R = H; R^1 = b (heminine)
5. R = Ac; R^1 = b

thus confirming the point of attachment of the sugar chain to C-3 hydroxyl group of the aglycone. The mass ion fragment at *m/z* 478 corresponded to monoglycoside, which was generated by the elimination of the terminal sugar S_2 from $[M]^+$. It was further fragmented to give mass ion peak at *m/z* 373 [genin + $K]^+$ and *m/z* 145 [S_1 − OH] confirming the sequence of the sugar as calogenin-D-oleandrose-3-*O*-methyl-D-fucose. The FAB mass spectrum also contained other relevant mass ion peaks of the genin and sugar moieties.

In light of the foregoing evidence, the structure of **1** was established as calogenin 3-*O*-3-*O*-methyl-β-D-fucopyranosyl-(1 → 4)-*O*-β-D-oleandropyranoside.

Heminine (**4**) {mp 132°C, $[\alpha]_D$ − 62.5°, (*m/z* 647 [M + $K]^+$ positive ion FABMS)} gave characteristic colour tests in Xanthydrol (Khare et al., 1984) and Keller–Killiani (Khare et al., 1984) reactions for 2,6-dideoxy sugars and positive colour in Liebermann–Burchardt test (Abisch and Reichstein, 1960) for steroids indicating it to be a steroidal glycoside of 2,6-dideoxy sugar(s) which was confirmed by the presence of two anomeric carbon signals at δ 101.0 and 99.6 in the ^{13}C NMR spectrum and two anomeric proton signals at δ 4.54 and 4.40 as double doublets along with two secondary methyl doublets at δ 1.34 (*J* = 6 Hz) and 1.32 (*J* = 6 Hz) in the ^1H NMR of **4**.

The identification of aglycone and sugars along with their sequence in **4** were established by the acid hydrolyses (Rangaswami and Reichstein, 1949; Srivastav et al., 1994). The hydrolysate gave calogenin (Srivastava et al., 1982), D-cymarose and D-digitoxose by comparison with authentic samples ($[\alpha]_D$, PC, TLC) and as confirmed in **1** (Krasso et al., 1963; Eppenberger et al., 1966). The presence of D-cymarose being at the terminal end was also supported by a negative $NaIO_4$ test with **4**.

The ^1H NMR spectrum of **4** contained two sets of double doublets of one proton each at δ 4.54 (*J* = 9 Hz and 2 Hz) and 4.40 (*J* = 8 Hz and 2 Hz) which were assigned to the anomeric protons of D-cymarose (S_2) and D-digitoxose (S_1) in axial configuration (Allgeier, 1968) suggesting the presence of the 2,6-dideoxy synthesis moieties in a hexapyranose $^4C_1$ (D) conformation joined through β-glycosidic linkages (Allgeier, 1968). The ^{13}C NMR data (Table 2) of **4** were in agreement with the results deduced from the chemical degradations and the ^1H NMR spectrum. A relatively downfield appearance of the two anomeric carbons at δ 101.0 and 99.6 showed β-glycosidic linkages for the two monosaccharide units. The inter-glycosidic bond between D-cymarose (S_2) and D-digitoxose (S_1) was fixed as 1 → 4 by the glycosidation shift of C-4 signal of D-digitoxose (S_1) at δ 83.7.

A triacetate (**5**), prepared by acetylation of **4** with Ac_2O in pyridine at 100°C, also confirmed the derived structure and the position of linkage to the aglycone and inter-sugar linkage. The downfield shift of the H-20 methine proton of the genin at δ 5.37–5.30 (Srivastava et al., 1982) in **5** suggested that the sugar chain was glycosidically linked to C-3 of the aglycone and the downfield shift of H-3 (S_1) at δ 4.25–4.20 further confirmed the 1 → 4 linkage. ^1H NMR also contains the signals for the anomeric protons and other important signals at their usual shifts (see Table 1).

The mass ion fragments in the FAB mass spectrum of **4** substantiated the derived conclusions. The mass spectrum showed the highest mass ion peak recorded at *m/z* 647 corresponded to $[M + K]^+$ and a protonated molecular ion peak at *m/z* 609 $[M + H]^+$. The mass ion peak recorded at *m/z* 564 originated from the loss of side chain at C-17 $[M - 45]^+$ from *m/z* 609 indicating that the sugar chain was glycosidically linked to C-3 hydroxyl group of the calogenin. The loss of the terminal sugar (D-cymarose) from *m/z* 609 gave a mass ion peak at 464 which corresponded to the monoglycoside and which further fragmented to give mass ion fragments at *m/z* 335 [genin + $H]^+$ and *m/z*

131 [D-digitoxose − OH]$^+$. The fragment ion peak at *m/z* 275 was assigned to [disaccharide − OH]$^+$ was also in agreement of the derived structure of **4**.

On the basis of above spectroscopic evidence and chemical transformations, the structure of heminine was deduced as calogenin 3-*O*-β-D-cymaropyranosyl-(1 → 4)-*O*-β-D-digitoxopyranoside.

3. Experimental

General procedures were the same as reported earlier (Deepak et al., 1997b). ^{1}H and ^{13}C NMR spectra were recorded with WM-400 MHz and AVANCE DRX 300 MHz Bruker spectrometers in $CDCl_3$ using TMS as internal standard. FAB mass spectra were recorded with JEOL mass spectrometer model JMS-SX-102 FAB with DA-6000 data system. Shade-dried stems (6 kg) of *H. indicus* were extracted and fractionated with solvents of different polarities as reported earlier (Deepak et al., 1997b). Sugars were detected by 50% H_2SO_4 on TLC and Vanillin-perchloric acid on PC. Repeated CC of the $CHCl_3$–EtOH (3:2) extract (2.2 gm) over silica gel using different polarities of $CHCl_3$–MeOH as eluent afforded denicunine (**1**) (49 mg) and heminine (**4**) (39 mg).

Denicunine (**1**). mp 148°C, $[\alpha]_D + 20°$ (*c*, 0.11, MeOH), found: C 65.83; H 9.14 $C_{35}H_{58}O_{10}$ requires C 65.80; H 9.15. It gave a violet colour in the Liebermann–Burchardt test, a pink colour with xanthydrol, a blue colour in the Keller–Killiani test and a crimson colour in the Feigl test. FABMS *m/z*: 677 [M + K]$^+$, 638 [M]$^+$, 623 [638 − CH_3]$^+$, 605 [623 − H_2O]$^+$, 593 [638 − $CHOHCH_3$]$^+$, 591 [623 − CH_3OH]$^+$, 587 [605 − H_2O]$^+$, 576 [591 − CH_3]$^+$, 573 [605 − CH_3OH]$^+$, 572 [587 − CH_3]$^+$, 561 [593 − CH_3OH]$^+$, 531 [576 − $CHOHCH_3$]$^+$, 517 [561 − CH_3CHO]$^+$, 513 [531 − H_2O]$^+$, 478 [638 − S_2]$^+$, 463 [478 − CH_3]$^+$, 431 [463 − CH_3OH]$^+$, 416 [431 − CH_3]$^+$, 413 [431 − H_2O]$^+$, 373 [genin + K]$^+$, 322 [638 − genin]$^+$, 305 [disaccharide − OH]$^+$, 289 [genin − $CHOHCH_3$]$^+$, 273 [305 − CH_3OH]$^+$, 255 [273 − H_2O]$^+$, 253 [289 − $2H_2O$]$^+$, 241 [273 − CH_3OH]$^+$, 229 [273 − CH_3CHO]$^+$, 223 [253 − $2CH_3$]$^+$, 197 [229 − CH_3OH]$^+$, 178 [S_2]$^+$, 162 [S_1]$^+$, 161 [178 − OH]$^+$, 145 [S_1 − OH]$^+$, 143 [161 − H_2O]$^+$, 129 [161 − CH_3OH]$^+$, 127 [145 − H_2O]$^+$, 111 [129 − H_2O]$^+$, 95 [127 − CH_3OH]$^+$.

Heminine (**4**). mp 132°C, $[\alpha]_D - 62.5°$ (*c*, 0.11, MeOH), found: C 67.05; H 9.26 $C_{34}H_{56}O_9$ requires C 67.08; H 9.27. It gave a violet colour in the Liebermann–Burchardt test, a pink colour with xanthydrol and blue colour in the Keller–Killiani test. FABMS *m/z*: 647 [M + K]$^+$, 609 [M + H]$^+$, 578 [M − $2CH_3$]$^+$, 575 [M − CH_3, $-H_2O$]$^+$, 564 [609 − $CHOHCH_3$]$^+$, 548 [578 − $2CH_3$]$^+$, 503 [548 − $CHOHCH_3$]$^+$, 471 [503 − CH_3OH]$^+$, 464 [M − S_2]$^+$, 453 [471 − H_2O]$^+$, 449 [464 − CH_3]$^+$, 435 [453 − H_2O]$^+$, 413 [449 − $2H_2O$]$^+$, 369 [413 − CH_3CHO]$^+$, 335 [genin + H]$^+$, 289 [genin − $CHOHCH_3$]$^+$, 275 [disaccharide − OH]$^+$, 274 [289 − CH_3]$^+$, 257 [275 − H_2O]$^+$, 253 [289 − $2H_2O$]$^+$, 243 [275 − CH_3OH]$^+$, 241 [274 − CH_3, $-H_2O$]$^+$, 239 [275 − $2H_2O$]$^+$, 225 [257 − CH_3OH]$^+$, 223 [253 − $2CH_3$]$^+$, 207 [239 − CH_3OH]$^+$, 199 [243 − CH_3CHO]$^+$, 145 [S_2 − OH]$^+$, 131 [S_1 − OH]$^+$, 127 [145 − H_2O]$^+$, 113 [131 − H_2O]$^+$, 95 [127 − CH_3OH]$^+$.

Mannich and Siewert hydrolysis of **1**. To a solution of crystalline **1** (30 mg) in Me_2CO (5 ml), conc. HCl (0.05 ml) was added at room temperature. After 2 days, the reaction mixture exhibited two new spots (TLC, 50% H_2SO_4). The less polar spot was identical in mobility with calogenin (**2**) while the polar spot was presumably the monoglycoside. Hydrolysis was complete in 4 days. (PC, TLC). Usual workup afforded calogenin (**2**) (12 mg) mp 198–201°C $[\alpha]_D - 49.5°$ (*c*, 0.2, MeOH) and two chromatographically pure sugars identified as D-oleandrose (4 mg) $[\alpha]_D - 13°$ (*c*, 1.2, H_2O) and 3-*O*-methyl-D-fucose (4.5 mg) $[\alpha]_D + 109°$ (*c*, 0.8, H_2O).

D-Oleandronic acid phenylhydrazide and methyl 3-O-methyl-α-D-fucopyranoside were prepared by known methods (Renkonen et al., 1959; Collins, 1987).

Acetylation of **1**. Compound **1** (4 mg) was acetylated with Ac_2O (0.5 ml) in pyridine (0.5 ml) at 100°C for 1 h, which after usual workup afforded triacetate (**3**) (4.2 mg). ^{1}H NMR: δ 4.96–4.88 (1H, *m*, H-20), 2.05, 2.03, 1.99 (9H, 3*s*, 3OAc).

Mild acid hydrolysis of **4**. To a solution of **4** (10 mg) in 1,4-dioxane (1 ml) 0.1 N H_2SO_4 (1 ml) was added and the solution was warmed for 30 min at 50°C. The hydrolysis was complete in 2 days. Usual workup gave calogenin (**2**) (3.5 mg) mp 199–201°C $[\alpha]_D - 50°$ (*c*, 0.15, MeOH) and two chromatographically pure sugars identified as D-cymarose (1.9 mg) $[\alpha]_D + 49.9°$ (*c*, 0.11, H_2O) and D-digitoxose (1.8 mg) $[\alpha]_D + 42.6°$ (*c*, 0.16, MeOH).

Very mild acid hydrolysis of **4**. To a solution of **4** (10 mg) in 1,4-dioxane (1.5 ml) 0.01 N H_2SO_4 (1.5 ml) was added and the solution was kept at room temperature. After 3 days the reaction mixture exhibited two new spots identified as D-cymarose and presumably a monoglycoside. The hydrolysis was complete in 5 days which after usual work up afforded calogenin (**2**) (4 mg) mp 199–201°C $[\alpha]_D - 50°$ (*c*, 0.15, MeOH) and two chromatographically pure sugars identified as D-cymarose (2.1 mg) $[\alpha]_D + 49.9°$ (*c*, 0.11, H_2O) and D-digitoxose (1.9 mg) $[\alpha]_D + 42.6°$ (*c*, 0.16, MeOH).

D-Cymaronic acid phenylhydrazide and D-digitoxonic acid phenylhydrazide were prepared by known methods (Krasso et al., 1963; Srivastav et al., 1994).

Acetylation of **4**. Compound **4** (4 mg) was acetylated with Ac_2O (0.5 ml) in pyridine (0.5 ml) at 100°C for 1

h, which after usual workup afforded triacetate (**5**) (4.4 mg). ^{1}H NMR: δ 5.37–5.30 (1H, *m*, H-20), 4.25–4.20 (1H, *m*, H-3, S_1), 4.00–4.05 (1H, *m*, H-4, S_2), 2.07, 2.05, 2.00 (9H, 3*s*, 3OAc).

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