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21α,26-DIHYDROXY-D:A-FRIEDOOLEANAN-3-ONE FROM *SALACIA RETICULATA* VAR. *DIANDRA* (CELASTRACEAE)

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Key Word Index—*Salacia reticulata*; Celastraceae; 21α,26-dihydroxy-D:A-friedooleanan-3-one; iguesterin; pristimerin.

Abstract—Carbon-13 NMR has been used to detect the position of angular methyl oxygenation and determine the structure of 21α,26-dihydroxy-D:A-friedooleanan-3-one, a new trioxygenated friedooleanane from *Salacia reticulata* var. *diandra* and the assignment has been confirmed chemically.

INTRODUCTION

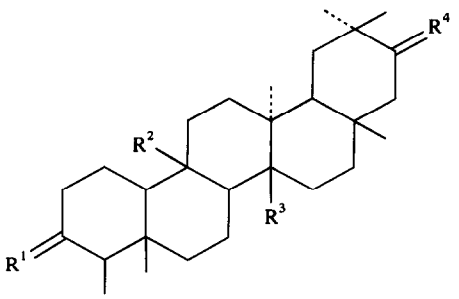
Salacia reticulata Wight is a woody climber, two varieties of which have been recognized in Sri Lanka, *S. reticulata* itself, growing in the submontane forests in the centre of the island and *S. reticulata* var. *diandra* growing in the low country rain forests of the south. Anthocyanidins, catechins, phenolic acids, quinones, friedooleananes, triterpene quinone-methides, mangiferin, gutta and dulctiol have been isolated from *Salacia* species [1–4]. The only work on *S. reticulata* has been the isolation of gutta, sitosterol, pristimerin and mangiferin from its root bark [5, 6].

We now report the structural elucidation of a new angular methyl oxygenated D:A-friedooleanane (**1**) from *S. reticulata* var. *diandra* using ^{13}C NMR chemical shifts and confirmation of the structure by chemical methods. Location of oxygen functions in angular methyl oxygenated triterpenes often requires tedious chemical transformations although there have been several reports of such compounds particularly in the oleanane and D:A-friedooleanane series [7]. The use of ^{1}H NMR in detecting the position of oxygenation is restricted by the near overlap of methyl signals in the spectra, the inability to detect any changes in the neighbouring protons as they are buried under the methylene envelope and the absence of α-protons since the methyl group is usually of a tertiary nature. ^{13}C NMR does not suffer from these disadvantages and in the case of the D:A-friedooleananes the chemical shifts of several derivatives have been assigned [8], and the literature data together with considerations of substituent effects and general chemical shift arguments can be used to determine the structures of new D:A-friedooleananes. The method may be of general use in the structural elucidation of such terpenes.

RESULTS AND DISCUSSION

The methanol soluble fraction of the benzene extract of *Salacia reticulata* var. *diandra* bark contained the triterpene quinone-methides, iguesterin (**2**) and pristimerin (**3**) together with diol **1** which appeared from spectral evidence to be a D:A-friedooleanane with a carbonyl and two hydroxyl groups. The diol **1** gave a diacetate **4** whose ^{1}H NMR spectrum showed a two proton singlet at δ4.45. The presence of this signal and intense peaks in the mass spectra of both compounds corresponding to the loss of $-CH_2OR$ functions suggested that one of the hydroxyl groups was a hydroxymethylene group. Double doublets with coupling constants of 5 and 11 Hz at δ3.70 and 4.93 occurred in the ^{1}H NMR spectrum of the diol **1** and the acetate **4** respectively and these signals resembled closely the H-21β signals in D:A-friedooleanane-21α-ols and their acetates [4]. 21α-Hydroxy-D:A-friedooleanan-3-one (**5**) and D:A-friedooleanan-21α-ol (**6**) show double doublets at δ3.69 and δ3.68 respectively and their acetates at δ4.93 and δ4.91 respectively, with coupling constants of 5 and 11 Hz in every case. The diol **1** was probably a 21α-hydroxy-D:A-friedooleanane with a carbonyl group and one of its methyl groups oxygenated as a CH_2OH moiety. Comparison of ^{13}C NMR chemical shifts of the diol **1** with those of 21α-hydroxy-D:A-friedooleanan-3-one (**5**) indicated that most of the carbons in the compound show similar chemical shifts (Table 1).

The most shielded chemical shift in the ^{13}C NMR spectrum was that of the C-4 methyl (C-23), δ6.8, suggesting the carbonyl group to be at C-3, the C-4 methyl being deshielded and appearing at δ13 in D:A-friedooleananes lacking a 3-oxo substituent [Kumar, V. and Wijeratne, D. B. T., unpublished results]. Further evidence for assigning the oxo group to C-3 is provided by the near coincidence of the chemical shifts of the ring A carbons, C-1–C-5, in compounds **1** and **5**. The ease in matching the chemical shifts of ring E carbons, C-19–C-22, is similarly additional evidence for the presence of the 21α-hydroxyl group in the diol **1**. Very little chemical shift change is seen in the two compounds for the 23,24,28,29 and 30 methyl carbons, precluding the hydroxymethyl group being at these positions. The CH_2OH group must be at C-9, C-13 or C-14. Comparison of ^{13}C chemical shifts of the hydroxymethylene carbon with those of 14-CH_2OH in D:A-friedooleanan-27-ols (δ64.4 ± 0.3) [9] suggests oxygenation at this position to be unlikely



1 $R^1 = O$; $R^2 = Me$; $R^3 = CH_2OH$; $R^4 = \alpha OH$, βH

4 $R^1 = O$; $R^2 = Me$; $R^3 = CH_2OAc$; $R^4 = \alpha OAc$, βH

5 $R^1 = O$; $R^2 = R^3 = Me$; $R^4 = \alpha OH$, βH

6 $R^1 = H_2$; $R^2 = R^3 = Me$; $R^4 = \alpha OH$, βH

7 $R^1 = O$; $R^2 = CH_2OH$; $R^3 = Me$; $R^4 = H_2$

8 $R^1 = \alpha OH$, βH; $R^2 = R^3 = Me$; $R^4 = H_2$

9 $R^1 = \alpha OH$, βH; $R^2 = R^3 = Me$; $R^4 = \alpha OH$, βH

10 $R^1 = O$; $R^2 = R^3 = Me$; $R^4 = O$

11 $R^1 = \alpha OH$, βH; $R^2 = Me$; $R^3 = CH_2OH$; $R^4 = H_2$

12 $R^1 = O$; $R^2 = Me$; $R^3 = CHO$; $R^4 = H_2$

Table 1. ^{13}C NMR chemical shifts of diol **1** and alcohol **5**

	1	5		1	5		1	5
C-1	22.3	22.3	C-11	35.4	35.3	C-21	74.3	74.3
C-2	41.4	41.5	C-12	23.4	30.4	C-22	46.6	47.0
C-3	213.0	213.2	C-13	39.6	39.0	C-23	6.7	6.8
C-4	58.1	58.2	C-14	42.7	38.8	C-24	14.4	14.7
C-5	42.2	42.1	C-15	30.0	30.2	C-25	17.9	18.2
C-6	42.4	41.2	C-16	36.3	36.1	C-26	63.2	17.7
C-7	20.7	18.2	C-17	32.9	32.5	C-27	20.2	19.3
C-8	51.2	51.5	C-18	44.9	44.3	C-28	32.7	33.1
C-9	37.5	37.5	C-19	36.0	36.0	C-29	24.9	24.9
C-10	59.9	59.5	C-20	34.5	34.0	C-30	32.0	31.9

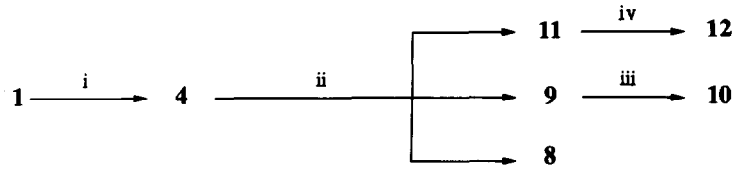
Further, any attempt to fit the data for 27-oxygenation results in an inexplicably large shielding (2–3 ppm) for the 25-methyl carbon. The ^{13}C NMR spectrum of 25-hydroxy-D:A-friedooleanan-3-one (**7**) shows perturbation of C-1 which is deshielded by 2.4 ppm in comparison with other 3-oxo friedooleananes and appears at 24.7 ppm [10]. The absence of any shift in the C-1 signal in the diol **1** from the 22.3 ppm value shown by the alcohol **5** suggests that oxygenation at C-25 too is unlikely leaving C-26 as the most probable site of oxygenation. The diol should therefore be 21α,26-dihydroxy-D:A-friedooleanan-3-one (**1**).

This assignment was shown to be correct by the deoxygenation of the diacetate **4** using lithium in ethylenediamine (Scheme 1). Deoxygenation gave D:A-friedooleanan-3α-ol (**8**) and two more polar products whose spectral characteristics indicated them to be diols. The less polar diol **9** on oxidation with Jones reagent gave D:A-friedooleanan-3,21-dione (**10**) [11] while the more polar diol (**11**) on oxidation with chromic acid–pyridine gave 3-oxo-D:A-friedooleanan-26-al (**12**) [12], the identity of both oxidation products being established by comparison with authentic material. These results confirm the oxygenation pattern deduced from ^{13}C NMR.

EXPERIMENTAL

Mps were determined on a Kofler hot stage apparatus and are uncorr. Identities of compounds were established by mmp, IR, mass and NMR comparison. Petrol refers to the fraction having boiling range 40–60° and prep. TLC was carried out on Merck Kiesel gel 60. Optical rotations were measured at 27° in $CHCl_3$. IR spectra were recorded for KBr discs, ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS as int. standard.

Isolation of 21α,26-*dihydroxy-D:A-friedooleanan-3-one* (**1**) *and triterpene quinone-methides* **2** *and* **3**. The bark of *Salacia reticulata* Wight var. *diandra* was collected from Kanneliya in Southern Sri Lanka and a voucher specimen deposited in the University herbarium. The outer bark (100 g) was separated, dried, ground and the powdered material extracted with C_6H_6 at 80°. Concn gave an orange solid (30 g) which was dissolved in $CHCl_3$. Addition of MeOH precipitated a cream powder (12 g) which was filtered off. The filtrate on concn gave a dark red solid (18 g) which was chromatographed on silica gel (400 g). Elution with



(i) $Ac_2O-C_5H_5N$, 27°, 18 hr (ii) Li (en), reflux, 30 min
(iii) $CrO_3-H_2SO_4$, Me_2CO, 27°, 3 hr (iv) $CrO_3-C_5H_5N$, 27°, 18 hr

Scheme 1.

$CHCl_3$ and MeOH–$CHCl_3$ (1:99) gave respectively iguesterin (**2**; 160 mg) as an orange amorphous solid, $[\alpha]_D$ −98° (lit. [13] mp 196–197°, $[\alpha]_D$ −99°) and pristimerin (**3**; 1.82 g), orange needles from MeOH, mp 213–214°, $[\alpha]_D$ −162° (lit. [14] mp 213–214°, $[\alpha]_D$ −164°) identical with authentic samples of iguesterin and pristimerin. Elution with MeOH–$CHCl_3$ (1:49) gave 21α,26-*dihydroxy-friedooleanan-3-one* (**1**) which recrystallized from petrol as colourless needles (280 mg) mp 271–273°, $[\alpha]_D$ −29°; Found: C, 78.2; H, 10.7. $C_{30}H_{50}O_3$ requires C, 78.6; H, 11.0%; IR ν_{max} cm^{-1}: 3500–3350; ^1H NMR: δ4.02 (2H, *dd*, *J* = 12 and 16 Hz), 3.70 (1H, *dd*, *J* = 5 and 11 Hz), 1.20, 1.16, 2 × 1.02, 0.94, 0.74 (each 3H, *s*) and 0.87 (3H, *d*, *J* = 7 Hz); MS *m/z* (rel. int.): 458 $[M]^+$ (1%), 427 (26), 409 (100) and 259 (62).

3-Oxo-D:A-friedooleanane-21α,26-diol, diacetate (**4**). Colourless needles from MeOH, mp 238–240°, $[\alpha]_D$ −7°; $[M]^+$ 542.3926. $C_{34}H_{54}O_5$ requires $[M]^+$ 542.3971; IR ν_{max} cm^{-1}: 1735, 1700; ^1H NMR: δ4.93 (1H, *dd*, *J* = 5 and 11 Hz), 4.45 (2H, *s*), 2.05, 2.00, 1.66, 1.26, 1.06, 0.94 × 2, 0.71 (each 3H, *s*) and 0.86 (*dd*, *J* = 7 Hz); MS *m/z* (rel. int.): 542 $[M]^+$ (12%), 482 (55), 469 (26), 422 (45) and 409 (100).

Lithium–ethylenediamine reduction of 3-oxo-D:A-friedooleanane-21α,26-diol, diacetate (**4**). The diacetate **4** (145 mg) in ethylenediamine (10 ml) was treated with Li (100 mg) under dry conditions until a blue colour appeared and the reaction mixture was kept at this temp for 20 min. It was then cooled and excess Li destroyed by addition of *t*-BuOH. The usual work-up gave a mixture of an alcohol and two diols which were separated by prep. TLC ($CHCl_3$–MeOH, 99:1, × 2). The alcohol **8** recrystallized from a $CHCl_3$–petrol mixture as colourless needles of D:A-friedooleanan-3α-ol (20 mg), mp 295–296°, $[\alpha]_D$ +16° (lit. [15] mp 292–201°, $[\alpha]_D$ +18°) identical with an authentic sample of D:A-friedooleanan-3α-ol. The less polar diol **9** recrystallized from a $CHCl_3$–petrol mixture as colourless needles (22 mg), mp 280–281°, $[\alpha]_D$ −21°; IR ν_{max} cm^{-1}: 3500; MS *m/z* (rel. int.): 444 $[M]^+$ (7%), 426 (31), 411 (15), 409 (21), 275 (33) and 257 (34); the more polar diol recrystallized from $CHCl_3$–MeOH as colourless needles (16 mg), mp 238–240°, $[\alpha]_D$ −12°; IR ν_{max} cm^{-1}: 3500–3300; MS *m/z* (rel. int.): 444 $[M]^+$ (1%) 426 (15), 413 (98), 395 (100), 291 (6) and 261 (40).

D:A-Friedooleanane-3,21-dione (**10**). Oxidation of the diol **9** (18 mg) with Jones reagent at 27° gave D:A-friedooleanane-3,21-dione (**10**), colourless needles from $CHCl_3$–MeOH, mp 248–250°, $[\alpha]_D$ +120° (lit. [11] mp 248–250°, $[\alpha]_D$ +115°) identical with an authentic sample of D:A-friedooleanane-3,21-dione.

3-Oxo-D: A-friedooleanan-26-al (**12**). Oxidation of the diol **11** (15 mg) with CrO_3 (12 mg) in pyridine (3 ml) during 18 hr gave on separation with prep. TLC ($CHCl_3$, × 2) 3-oxo-D:A-friedooleanan-26-al (**12**; 8 mg), colourless needles from petrol, mp 219–220°, $[\alpha]_D$ −30° (lit. [12] mp 224–225°, $[\alpha]_D$ −27°) identical with authentic material.

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