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**ARTICLE** *in* TETRAHEDRON · OCTOBER 2003

Impact Factor: 2.64 · DOI: 10.1016/j.tet.2003.08.063



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# Polyhydroxy pregnanes from *Dregea volubilis*

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Received 19 June 2003; revised 4 August 2003; accepted 28 August 2003

**Abstract**—Three new polyhydroxy pregnanes named dregealol (**1**), volubilogenone (**2**) and volubilol (**3**) were isolated from the flowers of *Dregea volubilis*, and their structures elucidated from extensive 2D NMR analysis. The structure of volubilol (**3**) was confirmed by X-ray crystallographic studies. The known pregnane derivatives drevogenin D, *iso*-drevogenin P and 17α-marsdenin were also isolated.


*Dregea volubilis* (Asclepiadaceae) is a woody climber, which occurs widely throughout the hotter parts of India.[1] In South East Asia, the plant is used in folk medicine as an antifebrile and emetic.[2] In a previous communication,[3] we reported the isolation and structure elucidation of three new pregnane glycosides, volubilosides A–C, and the identification of conduritol, quercetin and quercetin-3-*O*-rutinoside from the methanolic extract of its flowers. As pregnanes and pregnane glycosides are drawing much attention in recent years because of their antitumor and anticancer activities,[4] we continued with our study on the pregnane constituents of the plant. The present report deals with the isolation and characterization of three new polyhydroxy pregnanes, dregealol (**1**), volubilogenone (**2**) and volubilol (**3**), besides the known compounds *iso*-drevogenin P (**4**), 17α-marsdenin (**5**), and drevogenin D (**6**) from the less polar fraction of the methanolic extract. The stereochemistry and structures were determined mainly through the use of 1D and 2D NMR techniques. For **3**, the deductions were confirmed by X-ray crystallographic analysis.

## 1. Results and discussion

The molecular formula of dregealol (**1**) was determined as $C_{26}H_{30}O_6$ by HRFAB-MS (positive mode; found *m/z* 471.2713, calcd for $[M+Na]^+$ 471.2723) and $^{13}C$ NMR spectral data. The IR spectrum showed absorption bands at 3432 and 1686 $cm^{-1}$ for hydroxyl and carbonyl groups respectively. The $^{13}C$ NMR spectrum of **1** revealed the presence of five methyls, six methylenes, nine methines, and six quarternary carbons including signals appropriate for two trisubstituted double bonds and an ester function. From these results, coupled with the occurrence of pregnane glycosides in the plant, it was assumed that **1** possesses a five carbon side chain linked to a $C_{21}$ steroid skeleton. Assignment of proton and carbon resonances of **1** (Tables 1 and 2) was accomplished by extensive analysis of DEPT, $^1H-^1H$ COSY, and HMQC spectra. Comparison of the $^{13}C$ NMR spectrum with those of known pregnanes suggested that it could be an ester of drevogenin D. Its $^1H$ NMR spectrum showed an olefinic proton signal (δ 5.6, m) characteristic of $\Delta^5$-pregnanes. Another signal at δ 7.25 (dd, *J*=7.1, 1.6 Hz) coupled to an olefinic methyl signal (δ 1.64, 3H, dd, *J*=7.1, 0.9 Hz), along with an additional olefinic methyl singlet at δ 1.97 indicated the presence of a tigloyl moiety. This was fully supported by the observed HMBC correlation of olefinic carbon signals at δ 130.0 and 136.9 with the olefinic methyl proton signals. Mild alkaline hydrolysis of **1** yielded tiglic acid and drevogenin D. That the attachment of the tigloyl moiety was at C-20 could be inferred from the downfield shift of 1.46 ppm (from δ 4.17 to 5.63) observed for the H-20 signal along with upfield (4.1 ppm) shift for C-21 signal of drevogenin D, and substantiated by the HMBC correlation between signals for C-1′of the tigloyl group (δ 167.5) and H-20 (δ 5.63) of the pregnane moiety. From the foregoing evidences, the structure of dregealol (**1**) was determined as 20-*O*-tigloyl-drevogenin D.

*Keywords*: *Dregea volubilis*; polyhydroxy pregnane; dregealol; volubilogenone; volubilol; *iso*-drevogenin P; 17α-marsdenin; drevogenin D; 2D NMR; X-ray analysis.

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doi:10.1016/j.tet.2003.08.063

**Table 1**. $^{13}$C NMR chemical shifts ($\delta$) of compounds **1**–**6** in $d_5$-pyridine[a]

| C | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | 40.0 | 37.0 | 36.7 | 40.2 | 41.2 | 40.0 |
| 2. | 32.9 | 33.3 | 33.4 | 32.9 | 32.5 | 32.9 |
| 3. | 71.7 | 71.6 | 71.6 | 71.6 | 71.1 | 70.4 |
| 4. | 44.1 | 44.3 | 44.2 | 44.1 | 44.0 | 44.1 |
| 5. | 141.8 | 138.5 | 138.1 | 141.9 | 142.2 | 141.7 |
| 6. | 121.4 | 124.9 | 125.1 | 121.4 | 117.6 | 121.7 |
| 7. | 28.1 | 27.4 | 27.8 | 27.7 | 35.4 | 28.3 |
| 8. | 38.4 | 38.9 | 39.0 | 38.0 | 74.3 | 38.3 |
| 9. | 49.9 | 49.7 | 49.9 | 49.6 | 50.1 | 50.0 |
| 10. | 39.5 | 44.3 | 44.3 | 39.4 | 39.4 | 39.5 |
| 11. | 71.7 | 73.3 | 72.4 | 72.6 | 72.0 | 71.2 |
| 12. | 80.3 | 73.9 | 80.5 | 73.8 | 76.6 | 80.6 |
| 13. | 53.6 | 56.8 | 54.2 | 56.5 | 57.5 | 54.1 |
| 14. | 85.0 | 86.3 | 84.7 | 85.8 | 86.7 | 84.4 |
| 15. | 33.4 | 31.5 | 33.2 | 32.2 | 34.6 | 34.2 |
| 16. | 25.5 | 21.6 | 27.2 | 21.4 | 22.1 | 27.2 |
| 17. | 51.4 | 61.9 | 54.7 | 62.0 | 62.1 | 54.8 |
| 18. | 10.6 | 15.1 | 11.6 | 14.8 | 16.0 | 11.6 |
| 19. | 19.1 | 62.7 | 62.6 | 19.2 | 17.8 | 19.1 |
| 20. | 74.0 | 210.1 | 70.4 | 210.0 | 210.1 | 71.7 |
| 21. | 19.6 | 31.9 | 23.5 | 31.9 | 32.3 | 23.7 |
| 1′ | 167.5 | – | – | – | – | – |
| 2′ | 130.0 | – | – | – | – | – |
| 3′ | 136.9 | – | – | – | – | – |
| 4′ | 14.2 | – | – | – | – | – |
| 5′ | 12.3 | – | – | – | – | – |

[a] Assignments are based upon COSY, TOCSY, HETCOR, NOESY, DEPT and HMBC.

HRFAB-MS of **2** afforded a $[M+Na]^+$ peak at *m/z* 403.2081, suggesting the molecular formula to be $C_{21}H_{32}O_6$ (calcd 403.2097). Its IR spectrum showed absorption bands attributable to hydroxyl group and non-conjugated ketone. Analysis of $^1$H and $^{13}$C NMR spectra revealed the presence of two tertiary methyl groups ($\delta_H$ 1.87, $\delta_C$ 15.1 and $\delta_H$ 2.46, $\delta_C$ 31.9) assignable to C-18/C-19 and C-21 respectively. The downfield shift of the 21-$CH_3$ signal and the existence of a quaternary carbon peak at $\delta$ 210.1 in the $^{13}$C NMR spectrum suggested the presence of a keto group at C-20. In the $^1$H NMR spectrum, signals assignable to carbinylic protons were observed at $\delta$ 3.99 (m), 4.59 (t, $J$=10.0 Hz) and 3.87 (d, $J$=9.6 Hz), together with signals attributable to hydroxymethyl protons at $\delta$ 4.48 and 4.45 (each d, $J$=11.4 Hz). The carbinylic protons could be placed at 3α, 11β and 12α respectively, while the hydroxymethyl group could be at C-10 or C-13. In support of the above conclusions, five carbon signals were observed in the $^{13}$C NMR spectrum [$\delta$ 86.3, >C (OH)–; $\delta$ 62.7, –$CH_2$OH; $\delta$ 71.6, 73.3 and 73.9, 3×–CHOH]. The presence of the hydroxymethyl group at C-10 was established from the HMBC experiment, in which correlations were observed between 19-$H_2$ ($\delta$ 4.48 and 4.45) signals and those of C-1 ($\delta$ 37.0), C-5 (138.5), C-9 (49.7), and C-10 (44.3). The existence of NOESY relationship between H-11 ($\delta$ 4.59) and $H_3$-18 ($\delta$ 1.87) signals established the α-orientation of the 11-hydroxy group. The observed large coupling constant ($J$=9.6 Hz) of H-12 with H-11, coupled with the NOESY cross peaks noticed between signals of H-12 ($\delta$ 3.87) and H-9 ($\delta$ 1.66) suggested the equatorial configuration of the 12-hydroxy group. The orientation of the side chain at C-17 was established to be α as the 17-H signal ($\delta$ 3.53) showed NOESY relationship with 18-$CH_3$ signal; this was supported by the downfield shift of about 3–4 ppm for C-18 (absence of $\gamma_g$ effect) and upfield shifts ($\gamma_g$ effect) of 3 to 5 ppm for C-12 and C-15 compared to their 17β counterparts. From the above evidences, the structure of volubilogenone was established as 3β, 11α, 12β, 14β, 19-pentahydroxy-(17*S*)-pregn-5-en-20-one (**2**).

Volubilol (**3**) possesses the molecular formula $C_{21}H_{34}O_6$ based on the number of carbon atoms deduced from its $^{13}$C NMR (DEPT spectrum) and the presence of the sodiated molecular ion $[M+Na]^+$ peak at *m/z* 405.2253 in its mass spectrum (positive HRFAB-MS). The $^{13}$C NMR spectrum revealed the presence of a trisubstituted double bond besides one tertiary methyl, a hydroxymethyl, a secondary methyl, six methylenes, seven methines (five of them bearing an oxygen atom each), and three quaternary carbons (one of them bearing an oxygen atom). Comparing the $^{13}$C NMR signals of **3** with those of **2** and **6** (Table 1), it was concluded that the hydroxymethyl group is located at C-10 as in case of **2**. This was supported by the presence of HMBC correlations between 10-$CH_2$ ($\delta$ 4.46) signal and those of C-1 ($\delta$ 36.7), C-5 ($\delta$ 138.1), C-9 ($\delta$ 49.9) and C-10 ($\delta$ 44.3) signals. Absence of NOESY relationship between the signals of 17-H ($\delta$ 2.68) and 18-$CH_3$ ($\delta$ 1.84) suggested that the former must be 'α'-oriented. The configuration at C-20 is worked out to be *R* from the $^{13}$C chemical shift ($\delta$ 70.4).[5–7] Finally, the detailed structure and stereochemistry

**Table 2**. $^1$H NMR spectral data of **1**–**6** ($d_5$-pyridine, $\delta$),[a] coupling constant in parenthesis

| C | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 3 | 3.94 (m) | 3.99 (m) | 4.00 (m) | 3.93 (m) | 3.80 (m) | 3.94 (m) |
| 6 | 5.60 (m) (5.3) | 5. 85 (d) | 5.86 (m) | 5.58 (m) | 5.33 (m) | 5.60 (d) (4.8) |
| 8 | 2.18 (m) (4.6, 11.7) | 2.94 (dt) (4.6, 11.9) | 2.92 (dt) | 2.16 (m) | – | 2.25 (dt) (4.1, 11.6) |
| 9 | 1.69 (dd) (10.4, 12.1) | 1.66 (t) (11.3) | 1.71 (dd) (10.6, 12.1) | 1.64 (dd) (10.7, 11.9) | – | 1.69 (dd) (10.5, 12.2) |
| 11 | 4.15 (t) (10.0) | 4.59 (t) (10.0) | 4.69 (t) (10.0) | 4.08 (t) (10.0) | – | 4.21 (t) (10.0) |
| 12 | 3.61 (d) (9.4) | 3.87 (d) (9.6) | 3.64 (d) (9.7) | 3.82 (d) (9.4) | 3.86 (d) (9.5) | 3.59 (d) (9.1) |
| 17 | 2.90 (m) | 3.53 (t) (9.1) | 2.68 (m) | 3.52 (t) (9.0) | 3.25 (m) | 2.69 (m) |
| 18 | 1.51 (s) | 1.87 (s) | 1.84 (s) | 1.77 (s) | 1.95 (s) | 1.75 (s) |
| 19 | 1.39 (s) | 4.48, 4.45 (2xd) (11.4) | 4.46 (m) | 1.41 (s) | 1.78 (s) | 1.43 (s) |
| 20 | 5.6 (m) | – | 4.17 (quintet) (6.2) | – | – | 4.17 (m) |
| 21 | 1.38 (d) (6.4) | 2.46 (s) | 1.48 (d) (6.6) | 2.48 (s) | 2.39 (s) | 1.50 (d) (6.6) |
| *Tigloyl* | | | | | | |
| 3′ | 7.25 (dd) (1.6, 7.1) | | | | | |
| 4′ | 1.64 (dd) (0.9, 7.1) | | | | | |
| 5′ | 1.97 (s) | | | | | |

[a] Assignments are based upon COSY, TOCSY, HETCOR, NOESY, DEPT and HMBC.

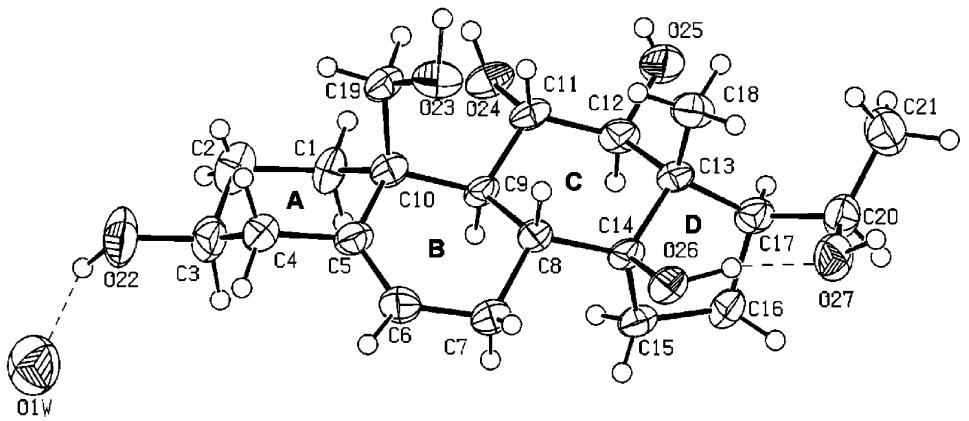



**Figure 1.** ORTEP-presentation of volubilol (**3**), the probability is 50%.

of **3** were established unambiguously from single-crystal X-ray diffraction studies. An ORTEP representation[8] of the molecule showing the atomic numbering scheme used and also the additional water oxygen is displayed in Figure 1. The molecule is composed of a tetracyclic ring system. Bond lengths and angles are normal for steroid type structure; the C-5, C-6 double bond was confirmed from the short bond length of 1.342 (9) Å. The six-membered rings A, B and C are *trans* fused among themselves, with the five membered ring D *cis* fused at C-13, C-14. The rings A and C are in the normal chair form as deduced from Cremer–Pople puckering parameters[9] (Table 3). Due to the double bond, ring B adopts an envelope form with C-8 as the out-of-plane atom. Ring D has an envelope conformation too, with C-14 as the out-of-plane atom. It is interesting to note that a rather strong intramolecular hydrogen bond is formed with the 14-β hydroxyl group (O-26-H) as donor, and O-27 of the –CHOH group at C-17 as acceptor. All other hydroxyl groups and the water molecule are involved in intermolecular hydrogen bonds (Table 4). Thus the structure of volubilol was established unambiguously as 17(*S*),20(*R*)-pregn-5-ene-3β,11α,12β,14β,19,20-hexol (**3**).

**Table 3**. Cremer Pople puckering parameters of the rings A to D for volubilol (**3**)

| Ring | $Q$, $q_2$ (Å) | $\Phi$, $\varphi_2$(°) | $\theta$ (°) | Type[a] |
|---|---|---|---|---|
| A | 0.567(7) | 148(7) | 6.2(7) | C |
| B | 0.490(6) | 196.9(9) | 54.6(7) | E |
| C | 0.559(6) | 187(13) | 3.3(6) | C |
| D | 0.374(6) | 34.0(1) | – | E |

[a] C=chair, E=envelope.

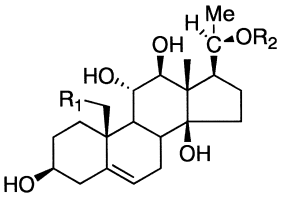
The molecular formula of **4** was found to be $C_{21}H_{32}O_5$ from the HRFAB-MS peak at *m/z* 387.2130 (calcd 387.2147 for $[M+Na]^+$). On comparing its $^{13}C$ NMR spectral data with those of drevogenin P reported by Abe et al.,[10] it was found that the signals for C-12, C-15, C-16 and C-20 shifted upfield, while those for C-17, C-18 and C-13 experienced downfield shift. Examination of the NOESY spectrum revealed relationship between the signals for 18-$CH_3$ ($\delta$ 1.77) and those of H-17 ($\delta$ 3.52), H-11β ($\delta$ 4.08) and H-8 ($\delta$ 2.16), indicating β-orientation of 17-H. In fact **4** was identified as *iso*-drevogenin P from its mp and $[\alpha]_D$ values[11] as well as from an analysis of the 1D and 2D NMR. As the $^{13}C$ NMR spectral data for *iso*-drevogenin P (**4**) was not reported earlier, these are being presented here (Table 1).

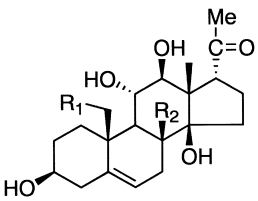
The molecular formula of **5** was deduced as $C_{21}H_{32}O_6$ from its high resolution positive ion FAB-MS (sodiated molecular ion at *m/z* 403.2199, 16 amu more than that of **4**). The $^{13}C$ NMR signals (Table 1) showed similarity to that of **4**, except for the possible presence of a hydroxy group at C-8 deduced from the downfield shift of the C-8 signal. Comparison of the $^{13}C$ NMR data with those of 17β-marsdenin reported by Umehara et al.[12] revealed significant differences in the chemical shifts for C-12, C-13, C-15, C-16, C-17, C-18, and C-20. Moreover, 17-H showed NOESY relationship with 18-$CH_3$. The compound was therefore concluded to be 17α-marsdenin. To the best of our knowledge, this is the first reported isolation of free 17α-marsdenin from a natural source. Saner et al.[11] had prepared this compound through alkali induced isomerisation of 17β-marsdenin, but the $^{13}C$ NMR chemical shift data are not yet available in the literature.

The molecular formula of **6** was determined as $C_{21}H_{34}O_5$ from the positive HRFAB-MS, which showed a quasi molecular ion peak at *m/z* 389.2297 $[M+Na]^+$ (calcd for $C_{21}H_{34}O_5Na$, 389.2304). Comparison of physical data and spectral data of **6** with those reported for drevogenin D in the literature[3] showed their identity.

It is worthwhile to mention that in C/D-*cis*-pregnanes, the application of $^{13}C$ NMR spectroscopy is very much helpful in establishing the C-17 configuration. Signal for C-18 is shifted downfield by about 4 to 5 ppm while those for C-12 and C-15 experience upfield shift of 3 to 4 ppm in 17*S* compounds compared to the 17*R* pregnanes, attributed to elimination or introduction of $\gamma_g$ effects as appropriate. Other prominent shifts noticed are for C-16 (3–4 ppm upfield) and C-17 (4–5 ppm downfield).

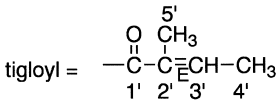
The compounds **1**–**6** were all tested for activity against HL-60 and U-937 leukemic cell lines.[15] No activity was observed even at a concentration of 100 μM in this assay.





**1**. Dregealol, $R_1$ = H; $R_2$ = tigloyl
**3**. Volubilol, $R_1$ = OH; $R_2$ = H
**6**. Drevogenin D, $R_1$= $R_2$ = H

**2**. Volubilogenone, $R_1$ = OH; $R_2$ = H
**4**. Iso-drevogenin P, $R_1$ = $R_2$ = H
**5**. 17α-Marsdenin, $R_1$ = H; $R_2$ = OH



## 2. Experimental

### 2.1. General

The plant material was collected from the suburbs of Kolkata, India, and was identified at Indian Botanic Garden, Howrah; a voucher specimen (No. 305) has been deposited at the Steroids and Terpenoids Chemistry Department of the Institute.

All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded as KBr pellets using a JASCO 7300 FTIR spectrometer. Optical rotations were measured with a JASCO DIP-370 polarimeter. The $^1$H and $^{13}$C NMR (1D and 2D) spectra were recorded at 500 MHz and 125 MHz respectively using a JEOL ECP-500 spectrometer in $C_5D_5N$ with TMS as internal standard. HRFAB MS (positive) were performed on a JEOL MS-700 mass spectrometer. Column chromatography was performed on silica gel 60 (Merck). TLC was carried out on precoated silica gel 60 F-254 plates, and spots were visualized by spraying with 10% $H_2SO_4$ followed by heating.

The defatted, air-dried powdered flowers (2.5 kg) of *D. volubilis* were extracted as described earlier[3] with methanol at ambient temperature, the extract concentrated under reduced pressure and extracted with water-saturated *n*-BuOH. The organic layer was evaporated under reduced pressure to give a residue (15.2 g), which was chromatographed on a column of silica gel using $CHCl_3$ and $CHCl_3$–MeOH (9:1, 17:3 and 4:1) successively. Earlier fractions eluted with $CHCl_3$–MeOH (9:1) on repeated chromatographic purification furnished dregealol (**1**, 87 mg), mixture of **4** and **5** (140 mg), drevogenin D (**6**, 280 mg), volubilogenone (**2**, 65 mg) and volubilol (**3**, 72 mg) according to increasing order of their polarity. Repeated fractional crystallization of the mixture of **4** and **5** from methanol-acetonitrile furnished *iso*-drevogenin P (**4**, 45 mg) and 17α-marsdenin (**5**, 20 mg).

**2.1.1. Dregealol (1).** The compound crystallised from methanol as microneedles, mp 184–186°C, $[\alpha]_D^{24}=-2.6$ (*c*, 1.0% in MeOH); IR: $\nu_{max}$ cm$^{-1}$ 3432, 2931, 1686, 1457, 1273, 1050 and 735; HRFABMS: *m/z* observed 471.2713, calcd for $C_{26}H_{40}O_6Na$, 471.2723; $^1$H and $^{13}$C NMR (Tables 1 and 2).

**2.1.2. Alkaline hydrolysis of 1.** Dregealol (10 mg) was refluxed with 5% methanolic KOH for 1 h. The reaction mixture was acidified with 5% HCl, extracted with $CH_2Cl_2$, and washed free from acid with water. The residue obtained after evaporation of the solvent was chromatographed over silica gel. Fractions eluted with $CHCl_3$-MeOH (99:1) furnished tiglic acid, whereas $CHCl_3$–MeOH (9:1) yielded drevogenin D.

**2.1.3. Volubilogenone (2).** The compound crystallized from methanol–acetonitrile as fine needles, mp 239°C, $[\alpha]_D^{24}=-74.0$ (*c*, 1.9% in MeOH); IR: $\nu_{max}$ cm$^{-1}$ 3404, 2928, 1689, 1358, 1207, 1039 and 820; HRFABMS: *m/z* observed 389.2297, calcd for $C_{21}H_{34}O_5Na$, 389.2304; $^1$H and $^{13}$C NMR (Tables 1 and 2).

**2.1.4. Volubilol (3).** The compound was crystallized from methanol as needles, mp 233–234°C, $[\alpha]_D^{24}=-8.36$ (*c*, 2.2% in MeOH); IR: $\nu_{max}$ cm$^{-1}$ 3396, 2928, 1469, 1293, 1042 and 821; HRFABMS: *m/z* observed 405.2245, calcd for $C_{21}H_{34}O_6Na$, 405.2253; $^1$H and $^{13}$C NMR (Tables 1 and 2).

*Crystal data of* **3**. $C_{21}H_{34}O_6{\cdot}1/2H_2O$, $M_r$=391.5, space

**Table 4**. Hydrogen-bonds for volubilol (**3**) (Å and °)

| D–H···A | *d*(D–H) | *d*(H···A) | *d*(D···A) | ∠(DHA) | Symm. |
|---|---|---|---|---|---|
| O22–H220···O1W | 0.98(8) | 2.02(9) | 2.958(6) | 159.(8) | *x,y,z* |
| O23–H23···O22 | 1.03(9) | 1.71(9) | 2.686(7) | 156.(8) | 1/2−*x*,1/2+*y*,1−*z* |
| O24–H24···O26 | 0.88(6) | 1.98(6) | 2.800(6) | 154.(4) | *x,y*,−1+*z* |
| O25–H25···O27 | 0.66(9) | 2.49(9) | 3.059(7) | 145.(6) | *x,y*,−1+*z* |
| O26–H26···O27 | 1.05(11) | 1.65(11) | 2.696(7) | 171.(11) | *x,y,z* |
| O27–H27···O23 | 0.91(8) | 1.84(8) | 2.744(6) | 173.(7) | 1/2−*x*,1/2+*y*,2−*z* |
| O1W···O25 | – | – | 2.792(6) | – | 1−*x*,2−*y,z* |

group orthorhombic $P2_12_12$. Lattice constants (Å) $a$=19.559(9), $b$=12.716(7), $c$=7.739(3), cell volume $V$=1924.8(16) Å$^3$, formula units/cell $Z$=4, X-ray density $\rho_x$=1.351 g cm$^{-3}$, number of independent reflections 1852, unobserved [$F_o<4\sigma(F_o)$] 401, $R_1$=0.059, $wR_2$=0.156, Gof=1.05.

*X-Ray experiments, structure determinations and refinements*. Colourless needle-shaped crystals of **3** were grown from methanol. The specimen used for X-ray experiments had dimensions of 0.65×0.05×0.03 mm$^3$. X-ray data collection was executed on a Stoe four circle diffractometer (Ni-filtered Cu Kα radiation) within an octand of reciprocal space ($h$,$k$,$l$, $2\theta\leq128°$) in the $\omega-2\theta$-scan modus with variable scan range and variable scan speed. Two standard reflections measured every 90 min showed no significant variations during the data collection.

Structure solution and refinement (programs SIR92[13] and SHELXL[14]) ran routinely. In addition to the title molecule, the oxygen atom of a water molecule was found, localised on the crystallographic two-fold axis. C, N and O atoms were refined anisotropically; isotropic displacement parameters were assigned to all the hydrogens, which could be located from a difference synthesis, except for the hydrogens of the crystal water molecule. After convergence of all parameters ($R_1$=5.9%, $R_w$=15.6%), no significant peaks or holes were seen in a final difference synthesis.

**2.1.5. *iso*-Drevogenin P (4).** It was crystallized from methanol as needles, mp 182–183°C, $[\alpha]_D^{24}=-40.3$ ($c$, 1.2% in MeOH); IR: $\nu_{max}$ cm$^{-1}$ 3414, 2928, 1699, 1359, 1043 and 814; HRFABMS: $m/z$ observed 387.2130, calcd for $C_{21}H_{32}O_5Na$, 387.2147; $^1$H and $^{13}$C NMR (Tables 1 and 2).

**2.1.6. 17α-Marsdenin (5).** Needle shaped crystals from methanol, mp 261–263°C, $[\alpha]_D^{24}=-10.35$ ($c$, 0.7% in MeOH); IR: $\nu_{max}$ cm$^{-1}$ 3410, 2921, 1680, 1437, 1242, 1050 and 818; HRFABMS: $m/z$ observed 403.2052, calcd for $C_{21}H_{32}O_6Na$, 403.2199; $^1$H and $^{13}$C NMR (Tables 1 and 2).

**2.1.7. Drevogenin D (6).** Fine crystals from methanol, mp 136–137°C, $[\alpha]_D^{24}=-4.36$ ($c$, 2.2% in MeOH); IR: $\nu_{max}$ cm$^{-1}$ 3384, 2929, 1458, 1106, 1049 and 814; HRFABMS: $m/z$ observed 389.2297, calcd for $C_{21}H_{34}O_5Na$, 389.2304; $^1$H and $^{13}$C NMR (Tables 1 and 2).

## 3. Supplementary material

CCDC 211194 (Deposition number) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336033; or deposit@ccdc.cam.ac.uk).

## Acknowledgements

N. P. Sahu is indebted to CSIR for award of Emeritus Scientist and N. Panda is thankful to DST for Junior Research Fellowship. The authors are also thankful to Dr J. R. Vedasiromoni, New Drug Development Division, Indian Institute of Chemical Biology for carrying out the studies against leukemic cell lines.

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