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# LIMONOIDS FROM THE SEED OF THE NATAL MAHOGANY, *TRICHILIA DREGEANA*

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**Key Word Index**—*Trichilia dregeana*; Meliaceae; limonoids; rohituka-7; dregeana-1 to 5; $^1$H NMR spectra; $^{13}$C NMR spectra.

**Abstract**—We have investigated the seed limonoids of *Trichilia dregeana* and obtained the known rohituka-7, an ester related to polystachin, and four new compounds designated dregeana-1 to 4. These were found to be intermediate in oxidation level between rohituka-7 and heudelottin.

## INTRODUCTION

*Trichilia dregeana* Sonder, the Natal mahogany, is a tree widespread in eastern Africa. We have previously described the isolation of dregeanin (**6**) from the timber of a Uganda specimen [1, 2], the bark of another gave a similar, unidentified compound (K. Pegel, personal communication). We now report the investigation of the seed. Only a small limonoid fraction was obtained, from this we isolated six pure compounds. One, which crystallized, was identified as the known rohituka-7 [3], the others which were poorly crystalline or amorphous, were new. The structures of all have been elucidated by physical methods.

## RESULTS AND DISCUSSION

Extraction of the minced seed with refluxing isohexane gave an oil (*ca* 50 %), which was partitioned between *iso*-hexane and methanol, giving a methanol-soluble fraction (1.25%), mixed triglycerides and palmitic acid.

Extraction of the seed coat separated from the cotyledons showed that it contained most of the limonoids, but the separation was very tedious. Chromatography of the methanol-soluble fraction over Si gel gave rohituka-7 [3] (0.01%) and a mother-liquor which after medium pressure chromatography [4] over Si gel gave five substances which we name dregeana-1 to dregeana-5.

A sample of the limonoid fraction was supplied to Mr. J. Akinniyi at Glasgow University, who obtained rohituka-7 and three of our five compounds by preparative TLC. We have not been able to repeat this, as in our hands preparative TLC produces changes in these compounds [3]. We do not know the reason for this difference.

Dregeana-1 remained amorphous. The NMR spectra were very similar to those of polystachin (**9**) [5], and it was recognised as the 4′-methyl-2′-hydroxy derivative (**1**). This variation in the 12-ester group is a very common one [3].

Dregeana-2 crystallized, but melted indefinitely at about 150°, and could not be recrystallized. The MS gave a molecular weight of 646.2674, corresponding to the formula $C_{33}H_{42}O_{13}$. The $^1$H NMR spectrum (Table 1) showed the presence of the usual furan ring, a carbomethoxy group and two acetates ($\delta$ 2.12, 2.06). A further methyl resonance (1.72) suggested a 12$\alpha$-acetate, shielded by the furan ring [6]. There was no resonance due to a formate. Double resonance experiments showed the presence of three coupled systems. The first corresponded to that previously observed in polystachin and similar limonoids of the prieurianin group for the coupled chain H-12$\beta$, H-11$\alpha$, H-9$\alpha$, 2H-30. This showed that dregeana-2 had 11$\beta$,12$\alpha$-acyl substituents and ring B fissioned. The second consisted of the characteristically wide multiplet due to H-17, coupled to 2H-16. The lack of further coupling suggested the presence of a carbonyl group at C-15. This was supported by the $^{13}$C NMR spectrum which had a ketonic carbonyl resonance at $\delta$ 206.5 and a singlet due to a carbon bearing a tertiary OH group at 83.8 which is characteristic of the 14-hydroxy-15-keto system in prieurianin derivatives. The third consisted of two coupled multiplets which we assign to H-1 and H-2 in a 1-acyloxy ring A lactone, also as found in prieurianin.

The $^{13}$C NMR spectrum showed the resonances expected for these structural features, and also for five ester carbonyl groups; one of these was the methyl ester, one the ring A lactone, and the other three were acyl groups which must be at C-1, C-11 and C-12. The molecular formula showed that these were all acetates, in agreement with deductions from the $^1$H NMR spectrum.

The $^1$H NMR spectrum showed resonances due to four tertiary methyl groups, and no resonance which could be due to a $CH_2$-O group. This meant that unlike all the previously known compounds in this series there was an unoxidized *gem*-dimethyl group at C-4, as in the simpler limonoids.

On the basis of this evidence we assign the structure **2**. This is unusual in having acetate residues at C-11, C-12 instead of the normal formate at C-11 and more complex ester at C-12. The resonance that we ascribe to H-1 ($\delta$ 5.5 $W_{1/2} = 15$ Hz) had a splitting greater than the usual range, corresponding values for analogous compounds are $\delta$ 5.2–5.8, $W_{1/2} = 6$–$12$ Hz [3]. We consider this is due

Table 1. $^1$H NMR spectra of *Trichilia dregeana* compounds

| Proton | **1** | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|
| 1 | 3.81<br>*m* (16) | 5.50<br>*m* (15) | 4.71<br>*m* (8) | 4.81<br>*m* (8) | 6.47<br>*d* (12) |
| 2 | — | 2.97<br>*m* | 3.12<br>*d* (4) | 3.23<br>*d* (3) | 5.95<br>*d* (12) |
| 7 | NA | NA | 5.26<br>*m* (6) | 5.27<br>*m* (6) | 5.33 |
| 9 | — | 3.72<br>*d* (7) | — | — | — |
| 11 | 5.40<br>*m* (15) | 5.22<br>*m* (18) | — | — | — |
| 12 | 6.13<br>*d* (12) | 5.93<br>*d* (11) | 5.06<br>*m* (17) | 5.03<br>*m* (18) | 5.19<br>*m* (16) |
| 15 | NA | NA | 5.47<br>*m* (5) | 5.56<br>*m* (6) | 5.52<br>*m* |
| 16 | — | 2.41, 2.89<br>*m* *m* | 2.41<br>*m* | 2.50<br>*m* | — |
| 17 | 3.97<br>*m* (20) | 3.93<br>*m* (20) | 2.93<br>*m* (17) | | |
| 29A | 4.14<br>*br. s* | NA | NA | 4.1<br>*d* (3) | 4.13<br>*d* (12) |
| 29B | 4.14<br>*br. s* | NA | NA | 4.0<br>*d* (3) | 4.58<br>*d* (12) |
| 30A | 5.51<br>*s* | 5.75<br>*br. s* | NA | NA | NA |
| 30B | 5.51<br>*s* | 6.05<br>*br. s* | NA | NA | NA |
| α furan A | 7.22 | 7.21 | 7.15 | 7.15 | 7.23 |
| α furan B | 7.36 | 7.32 | 7.31 | 7.31 | 7.35 |
| β furan | 6.22 | 6.23 | 6.21 | 6.21 | 6.28 |
| $CO_2Me$ | NA | 3.74 | NA | NA | NA |
| Ac | NA | 2.12<br>2.06<br>1.72 | 2.2<br>2.13<br>2.0 | 2.13<br>2.0 | 2.03 |
| CMe | 1.97<br>1.00<br>0.88 | 1.62<br>1.56<br>1.52<br>1.02 | 1.57<br>1.55<br>1.32<br>1.27<br>1.05 | 1.46<br>1.30<br>1.25<br>1.08 | — |
| 2′ | 3.38<br>*d* (3) | NA | 4.92<br>*d* (4.5) | 4.87<br>*d* 4.5<br>3.90<br>*br. s* | 4.03<br>*br. s* |
| Formate | 8.07<br>*s* | NA | NA | NA | NA |

Determined in $CDCl_3$ at 40° at 80 MHz, in ppm from internal TMS. Couplings, $J$ for doublets, $J_{AX} + J_{BX}$ for ABX systems, in Hz are given in parentheses. NA = not applicable.

to the unknown stereochemical consequences of the lack of oxidation at C-29, and the replacement of a formate at C-11 by an acetate. Ring A is attached to the rest of the molecule by only the C-9, C-10 bond, about which it can rotate [3]; and therefore it must be expected to show some conformational freedom.

Dregeana-3 also solidified on standing, and then melted indefinitely about 100°. The MS gave a molecular weight of 684.3538, corresponding to the formula $C_{38}H_{52}O_{11}$. The $^1$H NMR spectrum was not similar to those of prieurianin and related compounds, but instead resembled those of the heudelottins (**8**) [7]. The $^1$H NMR spectrum (Table 1) showed the usual furan bands, three bands due to acetate resonances, and five downfield resonances due to one vinyl proton and four that were vicinal to acyloxy groups. The $^{13}$C NMR spectrum showed lines due to the furan ring and one extra double bond (δ 156.0*s*, 122.1*d*), five ester carbonyl groups and five acyloxy carbons.

One of the downfield proton resonances was a doublet (δ 4.92, $J$ = 4.5 Hz). This we assign to H-2′ in a 2′-acetoxy-3-methylvalerate residue [3]. The others were all the X parts of ABX systems. The first (δ 5.47, $W_{1/2}$ = 5 Hz) was coupled to a 2 H multiplet (δ 2.41), which collapsed to a doublet on irradiating at 5.47. We assign this to a vinyl H-15 in a carbocyclic ring D. The second (5.26, $W_{1/2} = 6$ Hz) could not be decoupled. This we assign to H-7 in a 7α-acyloxy structure [7]. The fourth (4.71, $W_{1/2} = 8$ Hz) was coupled to a 2 H doublet (3.12, $J = 4$ Hz), which was not further coupled. This was assigned to H-1 in a ring A lactone of the nomilin type with the coupled doublet representing 2 H-2. The third (5.06, $W_{1/2} = 17$ Hz) could be due only to H-11 or H-12 if the structure was a conventional meliacan type; in *Trichilia* compounds this has so far been H-11α or H-12β. H-11α is coupled to three other protons (H-9, 2 H-12) and is normally widely split (11β-acetoxy gedunin, five lines $W_{1/2} = 21$ Hz [9]). H-12β is coupled to two others, and although the splitting is still considerable, the anticipated pattern is simpler (methyl 6,12α-diacetoxy angolensate, four lines, $W_{1/2} = 18$ Hz [6]). This suggests that the present three line resonance absorption may be due to H-12β, with a 12α-acyloxy group. Further evidence supporting this conclusion will be introduced in the discussion of substance **5**.

The methyl resonance of a 12α-acetate group is strongly shifted upfield, as in dregeana-2. Since this was not observed in the present case we assign two of the acetates to 7α and 1α and the acetoxy ester to 12α, giving the structure **3**.

Two substances closely related to **3** were also isolated. The first of these had NMR spectra very similar to those of **3** except for two bands, at $\delta$ 1.32 ($^{1}$H), and 29.5*t* ($^{13}$C). Both these indicate many methylene groups. The MS showed a molecular weight of 936, equivalent to the addition of 18 methylene groups to substance **3**, which suggested the presence of a molecule of esterifying fatty acid. The second similar substance (isolated by Mr. J. Akinniyi in Glasgow) was deacetyl-**3**, in which the side chain acetate was missing. The $^{1}$H NMR spectrum was very similar, the doublet at $\delta$ 4.92 was replaced by one at 4.05, the acetate band at 2.2 was missing, and the methyl resonances were slightly shifted.

Substance **4** remained amorphous. The MS molecular weight was 758.3876, corresponding to the formula $C_{41}H_{58}O_{13}$. The NMR spectra were similar to those of **3**, but indicated oxidation of one of the methyl groups to a hydroxy methylene (2 H, second-order structure, $\delta$ 4.10, 4.00 $J$ apparent = 3 Hz; $^{13}$C NMR: 66.2*t*). There were only two acetate resonances, and there was a new resonance at 3.90 (1 H, *br. s* ). The formula indicated the presence of 2-hydroxy-3-methyl butyric acid, which occurs at C-7 in the heudelottins (**8**) [7], as the acyl group in place of acetate. Acetylation of the 2′-hydroxy group in a 12-hydroxy ester causes a downfield shift of a furan resonance by about 0.1 ppm (**7**, $\delta$ 7.25, **7**-acetate, 7.37) [3]. Since there was no similar shift between **3** and **4** the hydroxy ester acetylated in **4** was that at C-12 and not that at C-7. Therefore structure **4** was assigned.

Acetylation gave a diacetate in which the 2H-29 resonances were shifted downfield ($\delta$ 4.86, not clearly resolved). The resonance at 3.90 (H-2′ in the hydroxy acid), also presumably moved downfield, but was not found in the diacetate.

It is a very curious fact that 2-hydroxy-3-methyl valeric acid is of common occurrence, often acetylated, at C-12 in *Trichilia* limonoids, while the closely similar 2-hydroxy 3-methyl butyric acid has only been found, but never acylated, at C-7 in the rather rare compounds where ring B has not been opened.

Dregeana-5 was obtained in very small amount, insufficient for a $^{13}$C NMR spectrum. The $^{1}$H NMR spectrum (Table 1) suggested that it differed from **4** only in the elimination of the C-1 acetate to give an unsaturated lactone and thus has the structure **5**. It is possible that **5** was an artefact, but we think this unlikely in view of the mild isolation conditions.

Further consideration of the spectrum of **5** provides support for locating the acyl group at C-12 and not at C-11 in the three related meliacan derivatives **3**, **4** and **5**. The positions of the H-1 resonance in ring A unsaturated limonoids is very sensitive to substitution at C-11: (Cedrelone, $\delta$ 6.86; 11β-acetoxycedrelone, 7.45) (D. A. H. Taylor, unpublished observations). Since in **5**, the resonance of H-1 was at the same position as in obacunone we deduce that **5** was unsubstituted at C-11, confirming the deduction made while discussing dregeana-3 above.

Substances **3**, **4** and **5** are very similar to those isolated recently by Epe and Mondon [9] from plants of the Cneoraceae and are the only compounds so far known which are intermediate between the intact meliacans such as heudelottin (**8**) [7] and the complex compounds of the prieurianin group. As such they may be of importance in the biogenesis of this complex group of substances.

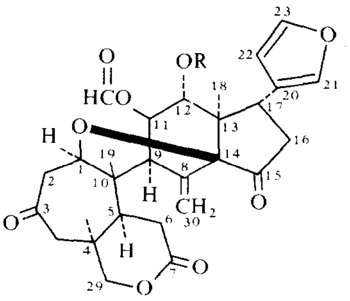
## EXPERIMENTAL

The seed of *Trichilia dregeana* (5.7 kg) collected from shade trees on the cricket ground of Durban High School; (herbarium specimens of one tree are deposited at the Forest Herbarium, Oxford as DAHT 299) was minced and then extracted by hot percolation with *i*-hexane. Evaporation of the solvent gave a greenish oil (*ca* 3 l.). This was diluted to about 7.5 l. with *i*-hexane and extracted 4 × with MeOH (95% aq., 1l. each time). These extracts were re-extracted with *i*-hexane (4 × 1l.) to give a 4 × 4 partition. The first two MeOH fractions seemed (TLC) to contain most of the limonoid and were combined and evapd to give a brown gum (70 g) which was the crude limonoid fraction. Fraction 3 gave a crystalline solid; a small sample of this was filtered off and washed with petrol, GLC of the derived methyl ester indicated that it was substantially pure palmitic acid.

The limonoid fraction was dissolved in EtOAc–hexane (1:3) and chromatographed on Si gel (1 kg). EtOAc–hexane (1:1) eluted the main limonoid fraction, which crystallized from MeOH giving rohituka-7 (520 mg). The mother liquor was evapd giving a residue (7 g) which was chromatographed over a succession of Si gel columns. Separation was monitored by TLC, only sufficient of each compound was isolated for recording the spectra. From the TLC it appeared that the seed contained roughly equal amounts of rohituka-7, and of substances **2** and **3**, with smaller amounts of substances **1** and **4** and trace amounts of **5** and of unisolated substances. NMR spectra were obtained after evapn of the samples with $CDCl_3$ to remove other solvents. $^{1}$H NMR spectra are collected in Table 1.

*Substance* **1** was amorphous, and gave an amorphous acetate with Py and $Ac_2O$. ($M^+$ 628, $C_{33}H_{40}O_{12}$ requires: 628. $^{13}$C NMR spectrum: $\delta$ 205.0*s*, 174.9*s*, 172.4*s*, 167.5*s*, 160.3*d*, 143.3*d*, 142.7*d*, 134.3*s*, 121.9*s*, 119.3*t*, 110.3*d*, 87.3*s*, 78.6*s*, 75.2*d*, 75.0*d*, 74.4*t*, 73.6*d*, 72.1*d*, 55.2*d*, 50.0*s*, 49.1*s*, 41.1*t*, 41.1*d*, 40.7*d*, 38.3*t*, 38.0*t*, 36.8*d*, 32.7*t*, 29.0*q*, 22.2*q*, 15.1*q*, 12.3*q*, 11.4*q*.)

*Substance* **2** crystallized on evapn of a MeOH soln, mp ~ 150° (not sharp). ($M^+$ 646.2674. $C_{33}H_{42}O_{13}$ requires: 646.2625. $^{13}$C NMR spectrum: 206.5*s*, 176.1*s*, 170.6*s*, 170.3*s*, 170.3*s*, 169.3*s*, 142.6*d*, 140.5*d*, 139.0*s*, 125.6*s*, 123.4*t*, 110.9*d*, 83.8*s*, 80.95*s*, 73.0*d*,



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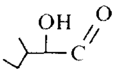
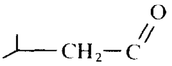
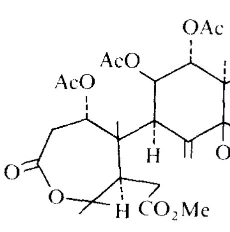


**9** R =.





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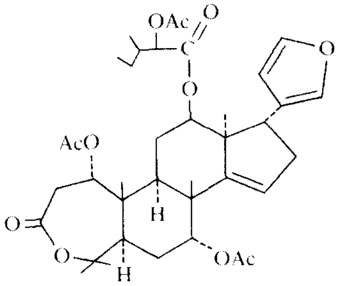

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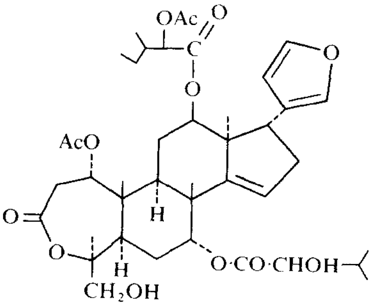

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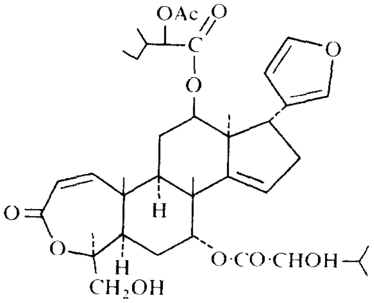

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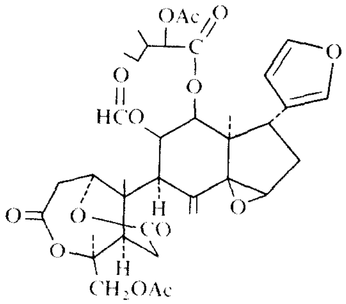

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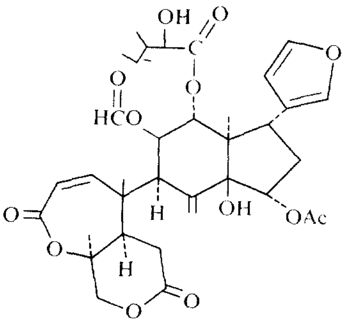

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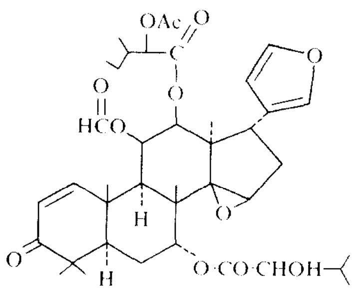

**8**

72.75*d*, 71.1*d*, 52.9*q*, 51.6*d*, 49.2*s*, 49.1*d*, 48.0*s*, 41.35*t*, 38.1*t*, 35.5*d*, 34.35*t*, 31.95*q*, 27.8*q*, 21.4*q*, 21.1*q*, 20.4*q*, 18.9*q*, 12.8*q*.)

*Substance* **3** also crystallized from a MeOH soln, mp. ~100° (not sharp.) ($M^+$ 684.3538, $C_{38}H_{52}O_{11}$ requires: 684.3589. $^{13}C$ NMR spectrum in $^{12}CDCl_3$: 170.7*s*, 170.7*s*, 169.9*s*, 169.9*s*, 169.7*s*, 156.0*s*, 142,2*d*, 140.4*d*, 125.2*s*, 122.1*d*, 111.7*d*, 85.4*s*, 76.5*d*, 75.1*d*, 74.9*d*, 71.1*d*, 51.4*s*, 50.2*d*, 44.4*s*, 44.1*d*, 41.7*s*, 37.5*d*, 37.3*d*, 36.8*t*, 35.2*t*, 34.1*t*, 29.7*t*, 29.2*q*, 26.4*t*, 25.0*q*, 23.9*q*, 23.9*q*, 21.2*q*, 20.6*q*, 15.0*q*, 15.0*q*, 14.6*q*, 11.5*q*.)

*Substance* **4** remained amorphous. ($M^+$ 758.3876, $C_{41}H_{58}O_{13}$ requires: 758.3877. $^{13}C$ NMR spectrum in $^{12}CDCl_3$: 174.8*s*, 174.0*s*, 170.9*s*, 169.6*s*, 168.8*s*, 156.0*s*, 142.6*d*, 140.8*d*, 124.7*s*, 122.7*d*, 111.9*d*, 85.4*s*, 77.2*d*, 76.3*d*, 75.8*d*, 75.6*d*, 71.1*d*, 66.2*t*, 51.8*s*, 50.6*d*, 44.8*s*, 44.8*d*, 42.0*s*, 39.5*d*, 37.6*d*, 37.2*t*, 35.7*t*, 32.2*d*, 29.3*q*, 28.3*q*, 26.8*t*, 26.0*t*, 24.4*t*, 21.4*q*, 20.8*q*, 19.5*q*, 16.3*q*, 15.6*q*, 15.1*q*, 11.8*q*.)

*Substance* **5** appeared to be pure, but remained amorphous. The quantity was inadequate for a $^{13}C$ spectrum.

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