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# A limonoid from *Neobeguea mahafalensis*

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**Abstract**

The hexane extract of *Neobeguea mahafalensis* has yielded a limonoid, neobeguin, which is related to pseudrelone A2. © 1999 Elsevier Science Ltd. All rights reserved.

*Keywords:* *Neobeguea mahafalensis*; Meliaceae; Limonoid; Neobeguin; Bussein

## 1. Introduction

*Neobeguea mahafalensis* Leroy. belongs to the *Neobeguea* genus of the Meliaceae family and is endemic to Madagascar. This plant is used medicinally in Madagascar (Pers. comm. to M. Randrianarivelojosia from indigenous user). A previous investigation of the jointly extracted wood and bark of this species yielded pseudrelone A2 **1** (Mulholland & Taylor, 1998). In this investigation, the hexane and dichloromethane extracts of the stem bark were investigated. Plant material was collected from the dry, thorny forests of the south of Madagascar and identified by M. Randrianarivelojosia. A voucher specimen (004 Mj/ M.Dul) is retained at the University of Antananarivo.

## 2. Results and discussion

The hexane and dichloromethane extracts of *N. mahafalensis* yielded β-amyrin and stigmasterol and the hexane extract also yielded neobeguin, **2**. High resolution mass spectrometry of **2** gave a molecular ion at *m*/*z* 714.2876 indicating a molecular formula $C_{37}H_{46}O_{14}$. Peaks at $[M\text{-}60]^+$ and $[M\text{-}120]^+$ indicated the loss of one and two molecules of acetic acid respectively suggesting the compound was a diacetate. The $^1$H NMR spectrum indicated that the compound was a limonoid with resonances at $\delta$ 7.48, $\delta$ 7.32 and $\delta$ 6.38 ascribable to H-21, H-23 and H-22 respectively of a β-substituted furanyl ring. A three-proton singlet at $\delta$ 3.68 indicated the presence of a methoxy group. In simple ring B-opened limonoids, ring opening results in a carbomethoxy group at C-7 and a terminal methylene group at C-30. No resonances ascribable to the two H-30 protons of the methylene group were present, indicating recyclisation had occurred to give a pseudrelone-type of compound as described previously from this source (Mulholland & Taylor, 1998). A bridged ring A system, a ring D-lactone and a recyclised pseudo-ring B with an orthoacetate system were shown to be present as in pseudrelone-type compounds. A pair of doublets at $\delta$ 1.81 and $\delta$ 1.89 in the $^1$H NMR spectrum were indicative of the non-equivalent H-29 protons of the 4, 29, 1-ring A bridge. The orthoacetate methyl group three proton resonance (3H-32) appeared at $\delta$ 1.56 and the carbon atoms of the orthoacetate group, C-31 and C-32, were present in the $^{13}$C NMR spectrum at $\delta$ 118.7 and 20.8 respectively, in accordance with literature values (Brown & Taylor, 1978; Connolly, Labbe & Rycroft, 1978). Resonances ascribable to H-3 and H-30 appeared as singlets at $\delta$ 4.85 and $\delta$ 5.33 respectively in the $^1$H NMR spectrum. As the COSY spectrum showed that

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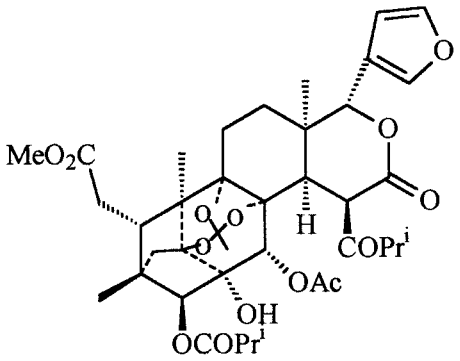

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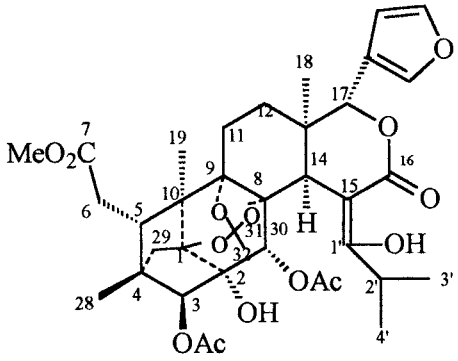

2

these protons were not coupled with any other protons, a hydroxy group was placed between them at C-2, as in pseudrelone A2, **1**. The presence of two acetate groups was confirmed by two three proton singlets at $\delta$ 2.26 and $\delta$ 1.96 in the $^1$H NMR spectrum and these were placed at C-3 and C-30 respectively.

The nature of substitution in ring C and at C-14 and C-15 in ring D remained to be determined. Fully substituted carbon resonances at $\delta$ 91.1 and $\delta$ 182.9 in the $^{13}$C NMR spectrum ascribable to C-1′ and C-15 respectively indicated a sidechain as in bussein A [2]. The H-2′ resonance occurred as a multiplet at $\delta$ 2.94 and 3H-3′ and 3H-4′ occurred as doublets at $\delta$ 1.24 and $\delta$ 1.11 respectively in the $^1$H NMR spectrum. The H-14 singlet occurred at $\delta$ 2.65. The C-11 and C-12 positions were not substituted and four coupled proton resonances at $\delta$ 2.02, 1.85, 1.48 and 1.18 could be assigned to the 2H-11 and 2H-12 protons. Thus, structure **2** was assigned to neobeguin. All $^1$H and $^{13}$C NMR data could be assigned using COSY, HETCOR and DELAYED HETCOR experiments. This compound differed from pseudrelone A2 in the ester present at C-3β. It is not clear from previous papers on pseudrelone A2 the exact nature of the substituent at C-15 as neither $^1$H nor $^{13}$C NMR data is given for H- or C-15 nor for C-1′ (Mulholland & Taylor, 1988; Ekong & Olagbemi, 1967).

There is much contradiction in the literature regarding the stereochemistry of these types of compounds. An X-ray structure was obtained for phragmalin, a related compound (Arndt & Baarschers, 1972), but contradictions within the paper and subsequent papers has led to confusion. On recyclisation of ring B to form the pseudo-B ring, rotation of ring A occurs around the C-9, C-10 bond in order for the new C-2, C-30 bond to form (Taylor, 1984). This results in H-3 and the 19-methyl group becoming α-orientated and H-5 and the 29-methyl group becoming β-orientated. The stereochemistry of H-30 and H-14 needed to be ascertained and that of H-3, H-5 and the methyl groups at C-18, 19 and 28 required confirmation. A series of NOE experiments was performed as illustrated in Fig. 1, confirming the stereochemistry of neobeguin as shown in **2**.

## 3. Experimental

Bark of *Neobeguea mahafalensis* (700 g) was extracted firstly with refluxing hexane and then with dichloromethane using a soxhlet apparatus to yield a hexane extract (36 g) and dichloromethane extract (45 g). A sample (7 g) of each extract was separated using column chromatography over silica gel (Merk 9385, solvent system 3/2/1, hexane/dichloromethane/ethyl acetate). Both extracts yielded β-amyrin and stigmasterol whose structures were confirmed by comparison against standard NMR data (Rubinstein, Goad, Clague & Mulheirn, 1976; Ahmad, 1976). The hexane extract also yielded **2**. $^1$H and $^{13}$C NMR data of **2** are given in Table 1.

*Neobeguin* (**2**) (230 mg), white crystalline, mp 228–230° (crystallised from MeOH). $M^+$ at $m/z$ 714.2876 (13%) ($C_{37}H_{46}O_{14}$ requires 714.2865), 654 $[M\text{-}60]^+$

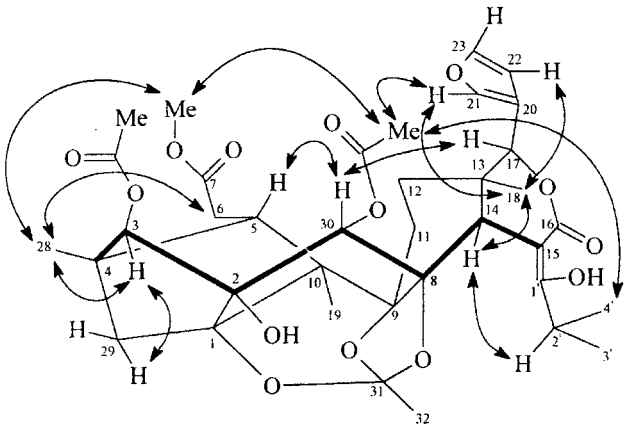

Fig. 1

Table 1
$^{1}H$, $^{13}C$ and NOE NMR data for **2** (300 MHz, $CDCl_3$, *J* in Hz in brackets)

| | $^{13}C$ | $^{1}H$ | NOE observed |
|---|---|---|---|
| 1 | 84.3 s | – | |
| 2 | 77.9 s | – | |
| 3 | 83.1 d | 4.85 s | 3H-28, H-29A |
| 4 | 40.1 s | – | |
| 5 | 37.2 d | 3.00 m | |
| 6 | 33.9 t | 2.45 m, 2.23 m | |
| 7 | 172.7 s | – | |
| 8 | 80.7 s | – | |
| 9 | 85.1 s | – | |
| 10 | 46.0 s | – | |
| 11[a] | 24.5 t | 2.02 m, 1.85 m | |
| 12[a] | 31.2 t | 1.48 m, 1.18 m | |
| 13 | 45.7 s | – | |
| 14 | 44.9 d | 2.65 s | H-2′, 3H-18 |
| 15 | 91.1 s | – | |
| 16 | 169.4 s | – | |
| 17 | 70.9 d | 5.84 s | H-30 |
| 18 | 21.8 q | 1.21 s | H-14, 2′, 21, 22 |
| 19 | 15.6 q | 1.14 s | no results |
| 20 | 122.3 s | – | |
| 21 | 141.1 d | 7.48 br s | |
| 22 | 110.0 d | 6.38 br s | |
| 23 | 142.7 d | 7.32 br s | |
| 28 | 14.7 q | 0.94 s | H-3, H-6A |
| 29 | 39.7 t | 1.89 d (6.7), 1.81 d (6.7) | |
| 30 | 74.5 d | 5.33 | H-5, H-17 |
| 31 | 118.7 s | – | |
| 32 | 20.8 q | 1.56 s | no results |
| 1′ | 182.9 s | – | |
| 2′ | 30.1 d | 2.94 m | |
| 3′ | 18.4 q | 1.24 d (6.7) | |
| 4′ | 20.6 q | 1.11 d (6.7) | |
| OCH3 | 52.1 q | 3.68 s | |
| $OCOCH_3$ (at C-3) | 170 4 s | – | |
| $OCOCH_3$ (at C-30) | 170.8 s | – | |
| $OCOCH_3$ (at C-3) | 20.6 q | 1.96 s | no results |
| $OCOCH_3$ (at C-30) | 21.1 q | 2.26 s | H-21, $OCH_3$, 3H-4′ |

[a] Resonances may be interchanged.

(19%), 594 $[M-120]^+$ (5%), 583 $[M-131]^+$ (59%), 481 $[M-233]^+$ (26%), 463 $[M-251]^+$ (18%).
IR $\nu_{max}$(NaCl) $cm^{-1}$: 3569, 1743, 1638. $[\alpha]_D = -51.78$ (*c* 0.0072 g/ml, $CHCl_3$).

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