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# Complete $^{1}H$ and $^{13}C$ NMR Assignments of Four Caesalpin Furanoditerpenes of *Caesalpinia bonducella*

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ABSTRACT: Four cassane furanoditerpenes, including the known α-caesalpin and caesalpin F, were isolated from the roots of *Caesalpinia boducella*. The $^{1}H$ and $^{13}C$ NMR spectra of all four compounds were completely assigned by using a combination of 2D NMR experiments, which included $^{1}H$–$^{1}H$ COSY, HMQC, HMBC and NOESY sequences. © 1998 John Wiley & Sons, Ltd.

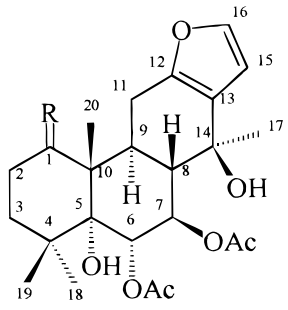
KEYWORDS: NMR; $^{1}H$ NMR; $^{13}C$ NMR; *Caesalpinia bonducella*; Caesalpiniaceae; cassane furanoditerpenes

## INTRODUCTION

*Caesalpinia bonducella* Flem. (Caesalpiniaceae) is a medicinal plant that is widely distributed throughout the tropics.[1–3] Initial investigations of the seed of this plant resulted in the isolation and structure elucidation of α-, β-, γ- and δ-caesalpin.[4–6] Further investigations of the seed resulted in the isolation and characterization of ε-caesalpin by $^{1}H$ NMR spectroscopy and x-ray analysis.[7] Subsequently, the stereochemistries of α-, β- and δ-caesalpin were determined by chemical interconversions and by comparison of their $^{13}C$ NMR spectra with that of ε-caesalpin.[8,9] More recently, caesalpin F was also isolated from the seed of *C. bonducella* and characterized by chemical transformations and $^{1}H$ NMR spectroscopy.[10] We have investigated the roots of *C. bonducella* and report here the isolation of α-caesalpin (**1**), caesalpin F (**2**) and two new furanoditerpenes, designated caesalpin G (**3**) and caesalpin H (**4**). The complete proton and carbon assignments and the relative stereochemistry of all four compounds were determined by the use of a series of 2D NMR experiments, which included $^{1}H$–$^{1}H$ COSY, HMQC, HMBC and NOESY sequences.

## RESULTS AND DISCUSSION

The $^{1}H$–$^{1}H$ COSY spectrum of **1** revealed the vicinal and geminal couplings, except for the coupling between H-6 and H-7. The $^{1}H$ NMR resonances due to H-6 and H-7 and also the $^{13}C$ signals for C-6 and C-7 were unusually broad; this suggested that this part of the molecule was conformationally mobile. This same broadening of the C-6/H-6 and C-7/H-7 signals were also observed in the $^{13}C$ and $^{1}H$ NMR spectra of **3** and **4** (see below). The one-bond $^{1}H$–$^{13}C$ connectivities were determined from an HMQC spectrum, whereas long-range correlations were established from an HMBC experiment. In the HMBC spectrum, the H-2 protons showed correlations with C-1 and C-3, and an H-3 proton at δ 1.92 correlated with C-1, C-2, C-4, C-5 and C-19. The HMBC spectrum also showed correlations between H-11β and δ 2.40 and the C-10 quaternary carbon and also C-9, C-12 and C-13. The stereo-




**1** R = O

**3** R = H, α-OH

**4** R = H, α-OAc

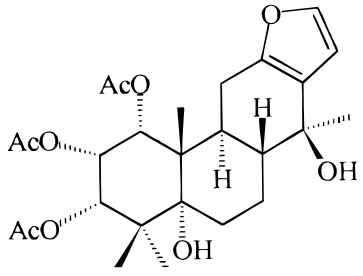



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**Table 1.** $^1$H NMR chemical shifts (ppm) and J($^1$H,$^1$H) couplings (Hz) of compounds 1–4

| Position | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | — | 5.25 (3.5) | 3.68 (7, 3) | 4.88 (t, 4) |
| 2α | 2.31 (16, 6, 5) | 5.49 (t, 3.5) | 1.68 | 1.76 |
| 2β | 2.79 (16, 10, 5) | | 1.97 | 1.91 |
| 3α | 1.92 | 5.17 (3.5) | 1.99 | 1.78 |
| 3β | 1.74 | | 1.09 | 1.12 |
| 6α | 5.57 (bs) | 1.87 | 5.54 (9) | 5.55 (9) |
| 6β | | 1.87 | | |
| 7α | 5.61 (bs) | 1.67 | 5.60 (bs) | 5.67 (bs) |
| 7β | | 1.77 | | |
| 8 | 2.10 | 1.75 | 2.21 | 2.20 |
| 9 | 2.84 (16, 10, 5) | 2.78 (13, 10, 6) | 2.99 (16, 12, 6) | 2.68 (13, 12, 5) |
| 11α | 3.15 (16, 5) | 2.23 (16, 6) | 2.78 (16, 6) | 2.31 (16, 5) |
| 11β | 2.40 (16, 10) | 2.48 (16, 10) | 2.51 (16, 11) | 2.49 (16, 11) |
| 15 | 6.35 (1.8) | 6.41 (2) | 6.38 (2) | 6.38 (2.2) |
| 16 | 7.22 (1.8) | 7.25 (2) | 7.24 (2) | 7.24 (2.2) |
| 17 | 1.48 | 1.35 | 1.51 | 1.52 |
| 18 | 1.13 | 1.14 | 1.10 | 1.16 |
| 19 | 1.32 | 1.26 | 1.16 | 1.18 |
| 20 | 1.52 | 1.25 | 1.19 | 1.29 |
| 1-Ac | — | 2.11 | — | 2.11 |
| 2-Ac | — | 1.97 | — | — |
| 3-Ac | — | 2.13 | — | — |
| 6-Ac | 2.00 | — | 2.00 | 1.99 |
| 7-Ac | 2.07 | — | 2.08 | 2.09 |

chemistry of **1** was established by examination of a NOESY spectrum. This spectrum was particularly useful for distinguishing H-2α from H-2β, H-3α from H-3β and H-11α from H-11β. The results of these experiments, which are summarized in Tables 1 and 2, led to the unambiguous assignment of all carbons and protons in α-caesalpin (**1**).

The structural characteristics of α-caesalpin (**1**) were established as early as 1963.[5] However, $^{13}$C NMR data, which were obtained in pyridine-$d_6$, were reported by Balmain *et al.*[8] for the caesalpins in 1981. The results now reported were determined in $CDCl_3$ solutions. There is very good agreement between the two sets of results; however, it is noticeable that many chemical shift values obtained in $CDCl_3$ are generally smaller than those determined in pyridine-$d_6$.[5] These differences are explainable in terms of the pyridine-induced shifts.

The $^1$H NMR spectrum of caesalpin F (**2**) had resonances due to three acetate oxymethine protons at δ 5.49 (t, $J$ = 3.5 Hz, H-2), δ 5.25 (d, $J$ = 3.5 Hz, H-1) and δ 5.17 (d, $J$ = 3.5 Hz, H-3), with corresponding carbons at δ 65.94, δ 73.94 and δ 77.10, respectively, as determined by HMQC. The couplings between these protons, which were also established from a $^1$H–$^1$H COSY spectrum, indicated that they were all β-oriented. This was also confirmed from a NOESY experiment. In the HMBC spectrum, H-1 showed long-range correlations to C-2, C-5 and C-10 and also an acetate carbonyl carbon at δ 169.41. On the other hand, H-3 showed long-range correlations to C-1, C-2, C-4 and C-5 and also an acetate carbonyl at δ 169.34, while H-2 showed correlation to an acetate carbonyl at δ 169.92. These results (Tables 1 and 2) indicated that the previous assignments for H-1 and H-3 in caesalpin F (**2**) should be reversed; this compound was previously isolated from the seed of a Jamaican sample of *C. bonducella*.[10]

Caesalpin G (**3**) was isolated as white crystals, m.p. 115–116 °C and had the molecular formula $C_{24}H_{34}O_8$. The IR spectrum exhibited absorptions due to hydroxy (3470 cm$^{-1}$) and ester (1744 cm$^{-1}$) functionalities. The $^1$H NMR spectrum of **3**, which was similar to that of **1**, had oxymethine resonances due to two acetates at δ 5.53 (bs) and δ 5.52 (bs) and a free hydroxy at δ 3.68 (dd, $J$ = 7.0, 3.0 Hz). This suggested that the ketone at C-1 in **1** was replaced by a hydroxy in **3**. The oxymethine proton at δ 3.68 was directly attached to a carbon at δ 72.60, as determined by inspection of its HMQC spec-

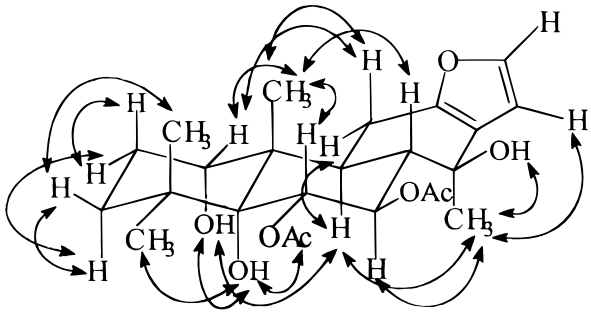


**Figure 1.** NOESY correlations for caesalpin G (**3**).

**Table 2.** $^{13}C$ NMR chemical shifts (ppm) of compounds 1–4

| Carbon | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | 211.63 | 73.94 | 72.60 | 76.66 |
| 2 | 35.18 | 65.94 | 25.41 | 21.94 |
| 3 | 39.36 | 77.10 | 31.80 | 32.10 |
| 4 | 38.80 | 43.14 | 38.56 | 38.41 |
| 5 | 82.76 | 76.62 | 80.33 | 79.05 |
| 6 | 74.80 | 19.00 | 75.44 | 74.91 |
| 7 | 73.07 | 26.09 | 74.10 | 73.68 |
| 8 | 48.09 | 43.50 | 47.51 | 47.56 |
| 9 | 36.76 | 36.14 | 35.31 | 35.67 |
| 10 | 54.95 | 45.40 | 44.45 | 44.47 |
| 11 | 25.21 | 21.86 | 22.56 | 22.55 |
| 12 | 149.14 | 148.43 | 148.24 | 147.46 |
| 13 | 124.26 | 125.13 | 125.02 | 125.30 |
| 14 | 72.87 | 71.96 | 72.73 | 72.48 |
| 15 | 107.10 | 107.34 | 107.24 | 107.29 |
| 16 | 141.56 | 141.54 | 141.74 | 141.84 |
| 17 | 24.46 | 25.09 | 24.96 | 24.85 |
| 18 | 29.02 | 23.16 | 30.38 | 30.50 |
| 19 | 25.62 | 25.48 | 24.75 | 24.89 |
| 20 | 16.44 | 18.21 | 17.07 | 17.16 |
| 1-Ac | — | 169.41 | — | 168.93 |
| | — | 20.93 | — | 21.37 |
| 2-Ac | — | 169.92 | — | — |
| | — | 20.70 | — | — |
| 3-Ac | — | 169.34 | — | — |
| | — | 21.14 | — | — |
| 6-Ac | 170.76 | — | 170.68 | 170.60 |
| | 21.46 | — | 21.50 | 21.42 |
| 7-Ac | 169.79 | — | 170.14 | 170.40 |
| | 21.47 | — | 21.65 | 21.58 |

trum. The HMBC spectrum revealed that H-1 correlated with C-3, C-5, C-10 and C-20. The stereochemistry of H-1 followed from its vicinal couplings to the C-2 protons and also from the interpretation of a NOESY spectrum (Fig. 1). These results are summarized in Tables 1 and 2 and led to the complete structural assignments for caesalpin G (**3**).

Caesalpin H (**4**), $C_{26}H_{36}O_9$, was isolated as white crystals, m.p. 114–115 °C. The $^1H$ NMR spectrum was similar to that of **3** except for the disappearance of the oxymethine proton due to a free hydroxy group at C-1 in **3** and the appearance of an acetate oxymethine at $\delta$ 4.88 (t, $J = 4.0$ Hz) in **4**. The HMQC spectrum showed that H-1 was directly bonded to a carbon at $\delta$ 75.66, while the HMBC spectrum indicated that it was long-range coupled to C-2, C-3, C-5, C-10 and C-20 in addition to an acetate carbonyl at $\delta$ 168.93. In the NOESY spectrum, H-1 showed correlation to the C-20 methyl, which established that it was $\beta$-oriented. The results of these experiments led to the structure **4** for caesalpin H (Tables 1 and 2).

## EXPERIMENTAL

### Product isolation

The roots of *C. bonducella* were collected in the Parish of St Andrew, Barbados, in February 1995. The dried, ground roots (1.7 kg) were extracted with 95% ethanol (9.6 l) and the solvent was evaporated *in vacuo* to give a brown, viscous syrup (180 g). The extract was dissolved in 90% methanol in water (500 ml) and extracted with light petroleum (6 × 300 ml). The aqueous methanol layer was diluted with water (200 ml) and extracted with dichloromethane (6 × 300 ml), dried over anhydrous sodium sulfate and the solvent evaporated to give a brown gum (24 g).

The dichloromethane extract was flashed chromatographed over silica gel with light petroleum–acetone (3:1) as eluent to give 13 major fractions. Fraction 9 (273 mg) was purified by HPLC on a preparative $C_{18}$ column using methanol–water (75:25), to give compounds **1** (28.0 mg) and **3** (11.6 mg), while fraction 8 gave **2** (237.0 mg) and **4** (165.5 mg).

**Compound 1.** Colorless crystals, m.p. 163 °C; $[\alpha]_D + 37.5°$ ($c = 0.40$, $CHCl_3$); IR ($CHCl_3$), $\nu_{max}$ 3392, 1747, 1710, 1602 $cm^{-1}$; UV (MeOH), $\lambda_{max}$ (log $\varepsilon$) 216 nm (3.87); EIMS, $m/z$ 448 ($M^+$, 4%), 433(21), 391(10), 371(15), 331(23), 310(100), 267(32), 241(64), 155(65), 124(80), 109(60), 97(18); HREIMS, 448.2095; calculated for $C_{24}H_{32}O_8$, 448.2097; $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2, respectively.

**Compound 2.** Colorless crystals, m.p. 230 °C, $[\alpha]_D + 0.7°$ ($c = 1.53$, $CHCl_3$); IR ($CHCl_3$), $\nu_{max}$ 3440, 1747, 1648, 1602 $cm^{-1}$; UV (MeOH), $\lambda_{max}$ (log $\varepsilon$) 212, 224 nm (3.77, 3.78); EIMS, $m/z$ 474 ($M^+$, 0.5%), 474(56), 456(48), 414(12), 336(15), 312(32), 279(84), 239(53), 162(68), 145(100), 131(22), 109(13); HREIMS, 474.2253; calculated for $C_{26}H_{34}O_8$ ($M - H_2O$), 474.2254; $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2, respectively.

**Compound 3.** Colorless crystals, m.p. 116 °C; $[\alpha]_D + 22.6°$ ($c = 0.13$, $CHCl_3$); IR ($CHCl_3$), $\nu_{max}$ 3470, 1744, 1674, 1602 $cm^{-1}$; UV (MeOH), $\lambda_{max}$ (log $\varepsilon$) 212 nm (3.08); EIMS, $m/z$ 450 ($M^+$, 5%), 435(28), 417(2), 375(36), 333(8), 312(53), 294(65), 231(38), 213(61), 189(56), 124(100), 109(73); HREIMS, 450.2263; calculated for $C_{24}H_{34}O_8$, 450.2254; $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2, respectively.

**Compound 4.** Colorless crystals, m.p. 114 °C, $[\alpha]_D + 17.9°$ ($c = 0.34$, $CHCl_3$); IR ($CHCl_3$), $\nu_{max}$ 3400, 1742, 1653, 1602 $cm^{-1}$; UV (MeOH), $\lambda_{max}$ (log $\varepsilon$) 216 nm (3.52); EIMS, $m/z$ 492 ($M^+$, 4%), 477(24), 432(5), 417(20), 375(11), 354(38), 312(42), 294(100), 243(47), 189(48), 124(66), 109(53); HREIMS, 492.2340; calculated for $C_{26}H_{36}O_9$, 492.2359; $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2, respectively.

### NMR spectra

All NMR spectra were obtained on a Varian UNITY 500 MHz spectrometer equipped with an inverse detection 5 mm probe ($^1H$ 90° pulse width = 9.5 μs, $^{13}C$ 90° pulse width = 9.4 μs). Samples contained 5–20 mg of a specific compound dissolved in *ca.* 1 ml of $CDCl_3$ with a trace of $(CH_3)_4Si$ added as an internal $^1H$ and $^{13}C$ reference. Initial assignments were with the aid of $^1H$ and $^{13}C$ spectra with a $^1H$ spectral width of 5000 Hz and a $^{13}C$ spectral width of 28 000 Hz, using 32K data points (zero filled to 64K) in each case. Complete assignments were made with the aid of COSY, HMQC and HMBC experiments. Typical COSY acquisition parameters were 3800 Hz for $f_1$ and $f_2$, 512 data points and 512 increments (both zero-filled to 1024), a 0.8 s relaxation delay and 16 transients per increment. HMQC and HMBC experiments used the same $^1H$ spectral window and respective $^{13}C$ spectral windows of 18 000 and 28 000 Hz, 1024 data points (zero-filled to 2048), 256 time increments, with linear prediction to 1024 and zero-filling to 2048 and a 0.7 s relaxation delay. Typical numbers of transients per increment for HMQC and HMBC were 16 and 64, respectively.

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