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ISOLATION AND STRUCTURE OF 15-O-BENZOYL-BRUCEIN D, A NEW QUASSINOID FROM SOULAMEA AMARA (X-RAY ANALYSIS)[1]

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Summary : The structure of 15-O-benzoyl-brucein D, a new quassinoid isolated from Soulamea amara Lam., has been established from spectral data and by single-crystal X-ray analysis ; the previously known picrasin B, isobrucein A and B were also isolated.

In the framework of our continuing studies on quassinoids[2] we examined those of Soulamea amara Lam., a Simaroubaceae indigenous to Vanuatu (New-Hebrides). Investigation of the aerial parts of this heretofore unevaluated Simaroubaceae showed that the major quassinoids present are the previously known picrasin B 1[3,4,5] and isobrucein A 2[5,6]. Among the minor components isolated are the known isobrucein B 3[7,8] and a novel quassinoid, 15-O-benzoyl-brucein D 4, the structural elucidation of which we report herein.

The dried ground aerial parts (1.5 kg) of Soulamea amara[9] were first extracted with hexane and then several times with hot water. The concentrated aqueous extract was continuously extracted with chloroform to give a crude mixture of products (14.1 g). Column chromatography of a part (4.1 g) of the chloroform residue on silica gel (Merck, 7734) and elution with chloroform containing 2 % methanol afforded pure picrasin B 1 (600 mg), a mixture of picrasin B and isobrucein A 2 (790 mg), pure isobrucein A 2 (370 mg), a fraction (275 mg) containing isobrucein A and a small amount of isobrucein B 3, and crude 15-O-benzoyl-brucein D 4 (220 mg). The latter was purified by preparative TLC (system : $CHCl_3$ + 10 % CH_3OH) to give pure 4 (110 mg).

Quassinoid 4 crystallized from methanol as colorless prisms, m.p. 296-298°, $[\alpha]_D^{22}$ + 24.05° (c = 1.0 ; pyridine). The molecular formula $C_{27}H_{30}O_{10}$ was established by high resolution FAB mass spectrometry with $(MH)^+$ at m/z 515.1948 (calculated : 515.1916). The CI mass spectrum (isobutane) showed abundant $(MH)^+$ and $(MH-18)^+$ ions and displayed a strong peak at m/z 123 assigned to protonated benzoic acid. The presence of a benzoyloxy

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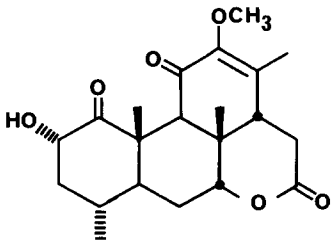

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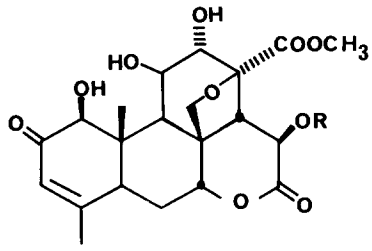
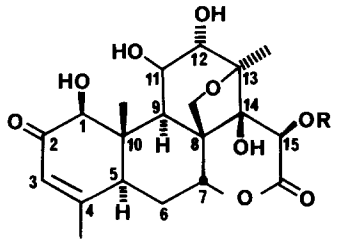
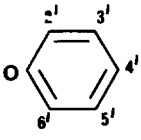


2 R = $COCH_2CH(CH_3)_2$

3 R = $COCH_3$





4 R = CO

5 R = H

group is also reflected by the EI spectrum which shows peaks at m/z 105 $(C_6H_5CO)^+$ and 77 $(C_6H_5)^+$. The i.r. spectrum (pyridine) showed carbonyl bands at 1665 (α,β-unsaturated ketone) and 1725 cm^{-1} (δ-lactone and benzoic ester), and the u.v. spectrum (EtOH) showed a maximum at 233 nm (ε = 20823) due to both the α,β unsaturated ketone and the benzoate moieties.

The 400 MHz ^{1}H-NMR spectrum (Fig.1) was particularly revealing. It showed the presence of three tertiary methyl groups at δ 1.24, 1.63 and 1.94 assigned to Me-13, Me-10 and Me-4, respectively. Extensive decoupling experiments allowed the identification of all the other resonances : δ 1.76 (1H, ddd, H_{6a}, J 2, 3, 12 Hz), 2.44 (1H, dd, H_{6e},

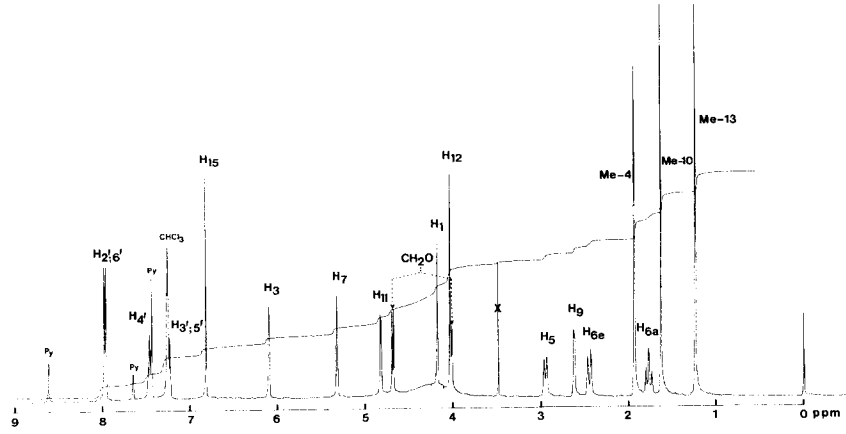


Fig. 1 : 400 MHz ^{1}H-N.M.R. spectrum of 4 in $CDCl_3$ - 10 % pyridine-d_5

2,12 Hz), 2.60 (1H, d, H_9, J 5Hz), 2.94 (1H, br.d, H_5, J 12 Hz), 4.00 and 4.67 (each 1H, AB quartet, $-CH_2O-$, J 7.5 Hz), 4.02 (1H, s, H_{12}), 4.16 (1H, s, H_1), 4.80 (1H, d, H_{11}, J 5 Hz), 5.31 (1H, t-like, H_7), 6.07 (1H, br.s., H_3), 6.79 (1H, s, H_{15}) and 7.2 to 7.92 (5H, aromatic protons). Except for the chemical shifts of H_{15} and of the aromatic protons these resonances are very similar to those of brucein D[10,11]. The oberved downfield shift for H_{15} suggests that the benzoyloxy group is located at position 15.

Unequivocal proof for the structure of quassinoid 4 was provided by single-crystal X-ray analysis.

A crystal of 4 grown from methanol, approximate size 0.4 x 0.3 x 0.6 mm, was mounted on a four circle automatic Philips PW 1100 diffractometer using graphite monochromatised Cu-Kα radiation (λ = 1,5418 Å). The system is monoclinic, space group $P2_1$ with two molecules in the cell (Z = 2). The parameters are : a = 11.178, b = 9.614, c = 11.810 Å and β = 114.6° with a total volume of 1153 $Å^3$. Each reflection was scanned over a 1° range at a speed of 0.025°. s^{-1}. Three standard reflections were scanned every two hours in order to check a possible decay in the data ; no decomposition was observed. Among a total of 2202 recorded reflections, 1945 with $I > 3\sigma(I)$ were considered as observed. Lorentz and polarisation factors were applied but no absorption corrections were made.

The structure was solved by the use of the multisolution techniques[12]. All atoms except three were found on the E map corresponding to the highest figure of merit. The three remaining atoms were readily obtained from Fourier recycling procedures with diffusion factors taken from the International Tables[13]. The refinements were performed by block diagonal least-squares procedures with isotropic, then anisotropic thermal factors for the heavy atoms and converged to $R = \Sigma \left||F_o| - |F_c|\right| / \Sigma |F_o| = 6.7$ %. Hydrogen atoms were placed at their theoretical positions with fixed isotropic thermal factors equal to the overall B factor which was determined at the normalisation stage of the resolution. They were not refined.

The molecular structure of 4 is shown in Fig. 2[14]. The ring junctions are the same as in brucein D 5 ; the configurations of the hydroxyl susbstituents are : 1β-OH, 11β-OH, 12α-OH, 14β-OH and the 15β-OH is esterified by benzoic acid. Ring A adopts an envelope conformation, rings B and C are in the chair form whereas the lactonic ring adopts a half-chair conformation. The dihedral angles between H-9 and H-11, and between H-11 and H-12 are 46.5° and 84.1°, respectively. These values are in agreement with the pattern and coupling constants observed for these protons in the ^{1}H-NMR spectrum of 4 (vide supra).

Quassinoid benzoates are rarely found in nature and only two have been isolated to date : bruceantarin[7] and dehydrobruceantarin[7].

Quassinoid 4 does not display any cell growth inhibition against the P388 lymphocytic leukemia cell line.

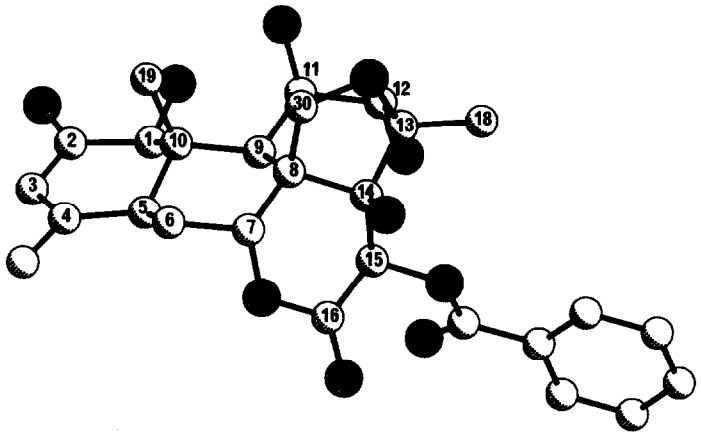


Fig. 2 : Molecular structure of 4 ; dark circles denote oxygen atoms

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