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ANTILEUKAEMIC QUASSINOIDS : STRUCTURE (X-RAY ANALYSIS) OF BRUCEINE C AND REVISED STRUCTURE OF BRUCEANTINOL

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Summary : The complete structure of bruceine C has been established by single crystal X-ray analysis of the tetra-O-acetyl derivative. The structure of bruceantinol, a potent antileukaemic quassinoid, has been revised and shown to be 4'-O-acetyl bruceine C.

Several quassinoids[1] have been shown to be promising antineoplastic agents[2,4] and in particular the 3,4-dimethyl-2-pentenoic acid ester of bruceolide[3] 1-bruceantin 2a[4] - has recently been placed on clinical trial by the US National Cancer Institute.

The first C-15 esters of bruceolide 1 to have their structures determined were bruceine A 2b, bruceine B 2c and bruceine C 2d, isolated in 1967 from *Brucea amarissima*[3]. The structural elucidation of the bruceine C ester side-chain at C-15 involved, in addition to spectral evidence (MS and ^{1}H-NMR), ozonolysis to give 3-hydroxy 3-methyl butan-2-one[3] ; the configuration of the double bond on the ester group was not established.

Later on, Kupchan *et al.*[4] isolated from *Brucea antidysenterica* several C-15 esters of bruceolide 1 among which two potent antileukaemic principles, bruceantin 2a and bruceantinol. The latter, different from bruceine C, was reported to be the C-15 *trans*-4-hydroxy-3,4-dimethyl-2-pentenoate ester of bruceolide on the basis of the nature of the products (the known bruceolide and methyl *trans*-4-hydroxy-3,4-dimethyl-2-pentenoate) obtained by alkaline hydrolysis followed by methylation.

The antileukaemic activity of the bruceolide derivatives varies greatly with the nature of the ester substituent[4,5]. It is therefore of great interest to establish the exact structure of the ester chains in bruceine C and bruceantinol thus contributing to the knowledge of structure-activity relationships in these series of cytotoxic quassinoids. We herein report the complete structural determination of bruceine C by X-ray analysis and also the revised structure of bruceantinol as shown in 2d and 2e, respectively. The partially acetylated derivatives of these quassinoids are described as well.

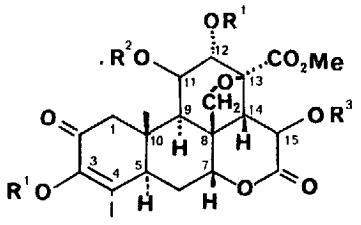
The ^{13}C-NMR spectrum[6] of bruceine C 2d confirmed the previously[3] proposed structure. The ^{1}H-NMR spectrum run now at 250 MHz revealed the expected signals for the methyl groups and double resonance experiments identified most of its signals (Table) . However, the configuration of the ester side chain double bond of bruceine C could not be assigned from these spectral data. Unequivocal proof for this configuration was provided by single X-ray analysis of 3,11,12,4'-tetra-O-acetyl bruceine C 4e, $[\alpha]_D^{20}$ + 19.6° (c 0.87, $CHCl_3$). Crystals of 4e

were obtained from methanol[7] : monoclinic, space group $P2_1$, a = 16.263 (4), b = 8.085 (5), c = 14.526 (4) Å and β = 103.94° (6). Direct methods[8] did not lead to conclusive results. The structural problem was solved using a Patterson search program[9] with the coordinates from the 6-hydroxypicrasin B[10] ring system. The best figure of merit was the starting point of Fourier recycling procedure. The structure was isotropically refined[11] to a final R factor = 13 %. The acetyl group of the side chain adopts two half weighted positions. No anisotropic refinement was performed because of the paucity of the data. The molecular conformation, shown in the Figure, confirms the structure 2d for bruceine C and establishes the *trans* configuration of its 2',3' double bond. The absolute configuration of bruceine C, also represented in 2d, follows from the experimentally proven triterpenoid biogenetic origin of the quassinoids.[1]

Structure 2d which is now firmly established for bruceine C was precisely that proposed for bruceantinol[4]. Since it was stated to be different from bruceine C,[4] the need for an unambiguous structure determination of bruceantinol became mandatory. We now show that bruceantinol is in fact 4'-O-acetyl bruceine C, 2e.

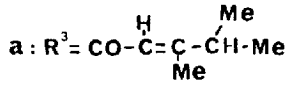
A sample of bruceantinol[12] was purified by preparative TLC to give ~ 10 % of a companion quassinoid (probably dehydrobruceantinol, λ_{max} 257 nm) and pure bruceantinol, $[\alpha]_D$ + 12°8 (c = 1.2 pyridine), λ_{max} 225 nm and 279 nm. Neither the electron impact mass spectrum nor the chemical ionisation (C.I.) spectrum[13] using isobutane as reactant gas ascertained the correct molecular weight of bruceantinol. By contrast, when the C.I. spectrum was run with ammonia, the $(MNH_4)^+$ ion peak was registered at m/e 624. The molecular weight of bruceantinol is therefore 606 which is 42 amu higher than that of bruceine C. The presence of an acetoxy group instead of a hydroxyl was therefore suspected from this mass difference. The location of the acetoxy group at the C-15 side chain ester is indicated by the presence in the (NH_3) I.C. spectrum of peaks at m/e 204 [$(CH_3)_2C(OAc)C(CH_3) = CHCO_2H$, NH_4]$^+$, m/e 186 (m/e 204-H_2O), m/e 144 (m/e 204-AcOH) and by a significant peak at m/e 456 [$(MNH_4)^+$-$(CH_3)_2C(OAc)C(CH_3) = C = CO$]. Comparison of the 250 MHz ^{1}H-NMR spectra obtained from bruceine C 2d, the peracetate derivative 4e with that of bruceantinol 2e revealed a substantial amount of structural information. By this means it was ascertained that only the C-15 ester side chain was modified and had an acetoxy group at C-4' instead of the hydroxyl. This finding was further substantiated by comparing the ^{13}C-NMR spectrum[6] of bruceine C 2d with that now obtained from bruceantinol 2e. These spectra showed near identity of the chemical shifts of most of the carbon atoms. That of bruceantinol 2e displayed two additional signals due to the acetoxy group (δ ~ 167 and 21.6 ppm) and its location at C-4' is consistent with the observed downfield shift for the C-4' resonance (82.2 ppm) with respect to that in bruceine C 2d (73.6 ppm).

Finally, the formation of identical acetyl derivatives from bruceine C and from bruceantinol 2e confirmed the structure of the latter. Acetylation (acetic anhydride/pyridine) of bruceine C 2d afforded the known[3] 3,11,12,4' tetra-O-acetyl bruceine C 4e, $[\alpha]_D$ + 19.6° (c = 0.87, $CHCl_3$) and 3,11,12-tri-O-acetyl bruceine C 4d, $[\alpha]_D$ + 22.4° (c = 1.06, $CHCl_3$). We have now isolated the new 3,12,4'-tri-O-acetyl bruceine C 3e, m.p. 250-251°C, $[\alpha]_D$ + 21.6° (c = 0.46, $CHCl_3$). The C.I. mass spectrum of this triacetyl derivative using isobutane as reactant gas reveals the MH^+ ion peak at m/e 691 and displays peaks at m/e 631 (MH^+-AcOH), m/e 589 (m/e 631-42) and m/e 565 (MH^+-3x42). The 250 MHz ^{1}H-NMR spectrum (Table) shows clearly that the



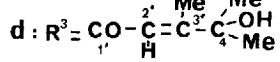
1 : $R^1 = R^2 = R^3 = H$ 2 : $R^1 = R^2 = H$

3 : $R^1 = Ac$; $R^2 = H$ 4 : $R^1 = R^2 = Ac$

a : $R^3 =$ 

b : $R^3 = CO-CH_2-CH(Me)_2$

c : $R^3 = Ac$

d : $R^3 =$ 

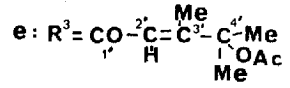
e : $R^3 =$ 

TABLE. 250 MHz ^1H NMR spectra of bruceine C 2d, bruceantinol 2e, 3,11,12,4'-Tetra-O-acetyl bruceine C 4e, 3,12,4'-Tri-O-acetyl bruceine C 3e and 3,11,12-Tri-O-acetyl bruceine C 4d in $CDCl_3$ [δ in ppm, J as (Hz)]

	2d	2e	4e	3e	4d
H-7	4.78 br.s	4.83 br.s	4.86	4.84 br.s	4.82
H-11	4.24 d(5)	4.28 t	5.22 d(4.5)	4.12 br.s	5.23 br.s
H-12	4.21 br.s	4.24 d(5)	5.23 br.s	5.30 br.s	5.28 br.s
$-CH_2O-$	3.78 d(8)	3.82 d(8)	3.85 d(7.5)	3.81 d(8)	3.83 d(8.5)
	4.72 d(8)	4.75 d(8)	4.75 d(7.5)	4.76 d(8)	4.73 d(8.5)
OMe	3.77	3.81	3.73	3.76	3.70
H-15*	6.22 d(10)	6.20 d(11)	6.22 d(11)	6.06 (10)	6.10 (11)
H-2'	6.07	5.78	5.75	5.76	6.04
Me-4	1.85	1.85	1.81	1.80	1.82
Me-10	1.38	1.40	1.31	1.50	1.28
Me-3'	2.17	2.14	2.14	2.14	2.17
Me-4'	1.37	1.53	1.54	1.54	1.34
Me	1.37	1.53	1.50	1.50	1.38
OAc		2.04	2.01 ; 2.02	2.01 ; 2.01	2.04 ; 2.12
			2.12 ; 2.25	2.26	2.24

* Appears as a distinct doublet when the spectra were measured at 50°C.

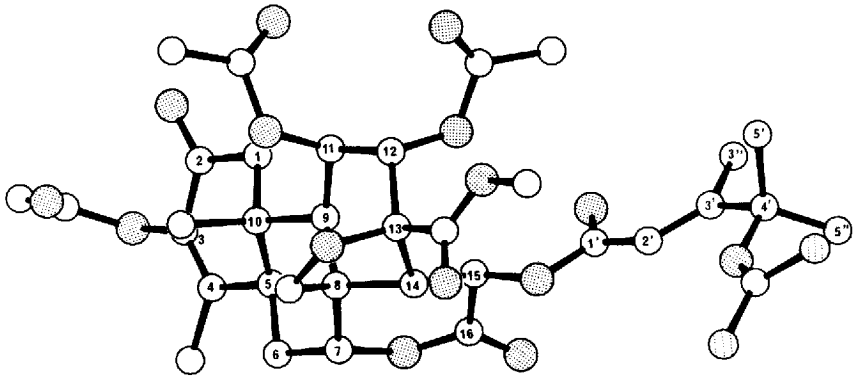


Figure. Molecular Structure of Tetra-O-acetyl bruceine C 4e

axial 11β-OH was not derivatised in 3e whereas the tertiary 4'-OH was acetylated (probably because it is situated vinylogously α to the ester group). Acetylation of bruceantinol 2e afforded 3,11,12-tri-O-acetyl bruceantinol and 3,12-di-O-acetyl bruceantinol which were found to be identical with 4e and 3e, respectively (by m.p., α_D, TLC, mass spectra and 250 MHz ^{1}H-NMR).

Bruceantin 2a and bruceantinol 2e showed potent antileukaemic activity against the murine P388 lymphocytic leukaemia 2a gave T/C values of 197 to 225 at doses of 0.5 to 2.0 mg/kg and 2e T/C values of 200 to 238 at doses of 0.25 to 2.0 mg/kg. Bruceine C 2d demonstrated lower activity in the same system and had an average T/C value of 152 at 1 mg/kg[14]. The markedly higher antitumor activity of bruceantin 2a and bruceantinol 2e versus bruceine C 2d could possibly be attributed to a greater lipophilicity in the side chain which may be a requirement for transport across cell membranes.[5]

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