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THE ISOLATION AND STRUCTURAL ELUCIDATION OF A NOVEL NATURALLY-OCCURRING BUFADIENOLIDE ORTHOACETATE[1]

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In the course of a continuing search for tumor inhibitors of plant origin, alcoholic extracts of Bersama abyssinica Fresen. (Melianthaceae) were found to show significant inhibitory activity against cells derived from human carcinoma of the nasopharynx (KB).[3,4] Our earlier report described the isolation and characterization of two cytotoxic principles, hellebrigenin 3-acetate and hellebrigenin 3,5-diacetate.[3] We report herein the isolation and structural elucidation of bersaldegenin 1,3,5-orthoacetate (1), a novel cytotoxic bufadienolide orthoacetate, and of bersaldegenin 3-acetate (7). Bersaldegenin 1,3,5-orthoacetate appears to be the first recognized naturally-occurring orthoacetate.

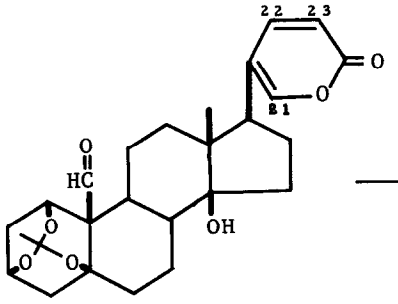
In this study, the concentrated ethanol extract of the stems (wood and bark) was defatted with petroleum ether and triturated with water. The aqueous solution was extracted with chloroform and with n-butanol. The cytotoxic residue from the chloroform extract was chromatographed on neutral alumina (Woelm, act.III). Elution with chloroform yielded bersaldegenin 1,3,5-orthoacetate (1); subsequent elution with 1% methanol in chloroform gave bersaldegenin 3-acetate (7).

Bersaldegenin 1,3,5-orthoacetate was crystallized from methanol as colorless plates, $C_{26}H_{32}O_7$; mp 288-295° d; $[\alpha]_D^{22}$ -24° (c 0.85, $CHCl_3$); M^+ m/e 456; λ_{max}^{MeOH} 298 mμ (ε 5050); λ_{max}^{KBr} 3.60, 5.80, 5.88, 6.11, 6.49, 7.10, 7.69, 8.85 μ; nmr signals[5] corresponding to two deshielded protons (at τ 5.25, 1H, br s, W_H = 6 Hz and τ 5.56, 1H, br s, W_H = 8 Hz), and a deshielded methyl group (τ 8.41, 3H, s), in addition to those indicative of bufadienolide, aldehyde, C-18 methyl, and hydroxyl functions.[cf.3] Although the IR and nmr spectra showed no bands characteristic of an acetate ester, the MS showed peaks at m/e M-43 and M-60, corresponding to the loss of acetyl and acetic acid units, respectively. This observation and the facts that the IR spectrum showed bands at 7.10 μ (orthoacetate displaced methyl)[6,7] and 8.85 μ (orthoacetate ether),[6,7] and the nmr spectrum showed a 3-proton singlet at τ 8.41 (orthoacetate methyl),[8] led to consideration of structure (1) for bersaldegenin 1,3,5-orthoacetate. Evidence for this structure was adduced from the experi-

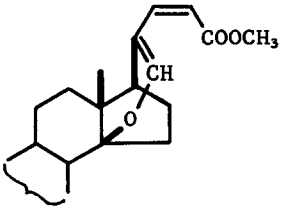
mental results which follow.

Treatment of bersaldegenin 1,3,5-orthoacetate with 80% aqueous acetic acid for 3 hr at 90-100° yielded a 1:1 mixture of 1 and a bersaldegenin monoacetate, $C_{26}H_{34}O_8$, mp 241-244° d; $[\alpha]_D^{22}$ ±0° (c 1.14, $CHCl_3$-MeOH, 5:1); M^+ m/e 474; λ_{max}^{MeOH} 297 mμ (ε 5190); λ_{max}^{KBr} 5.78 (br), 6.10, 6.45, 8.00 μ; nmr signals corresponding to an equatorial proton on acetate-bearing carbon (τ 3.82, 1H, br s, W_H = 6 Hz), an equatorial proton on hydroxyl-bearing carbon (τ 5.40, 1H, br s, W_H = 8 Hz), and an acetate methyl group (τ 8.04, 3H, s). Acetylation of the monoacetate with acetic anhydride-pyridine at 25° yielded bersaldegenin 1,3-diacetate (6), $C_{28}H_{36}O_9$; mp 255-258° d; $[\alpha]_D^{22}$ -14° (c 1.29, $CHCl_3$); M^+ m/e 516; λ_{max}^{MeOH} 298 mμ, (ε 4950); nmr signals corresponding to two equatorial protons on acetate-bearing carbons, at τ 3.91 (1H, br s, W_H = 5 Hz) and τ 4.55 (1H, br s, W_H = 8 Hz). The signal at τ 4.55 could be assigned to the C-3 proton, by comparison with the signal for the corresponding proton in hellebrigenin 3-acetate[3] (τ 4.65, 1H, br s, W_H = 8 Hz). The nature of the signal for the more deshielded proton in the diacetate 6 and the monoacetate was in accord with that expected for the C-1 proton in a 1-acetate ester. Consequently, the monoacetate was assigned structure 3.

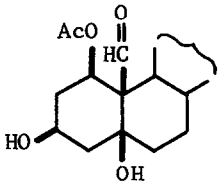
On the basis of biogenetic considerations and examination of Dreiding models of various possible locations of the orthoacetate, attachment at positions 1, 3 and 5 in ring A appeared likely. To test this hypothesis and, in addition, to secure information concerning the configuration of the orthoacetate, the synthesis of 3,19-cycloacetal derivatives was attempted. However, treatment of monoacetate 3 with 0.5% hydrogen chloride in absolute methanol for 2 hr at room temperature gave a quantitative yield of bersaldegenin 1,3,5-orthoacetate. Mild alkaline hydrolysis of 3 and 6 gave the new bufadienolide, bersaldegenin (4), $C_{24}H_{32}O_7$; mp 250-252° d; $[\alpha]_D^{22}$ -3° (c 0.73, $CHCl_3$-MeOH, 5:1); M^+ m/e 432; λ_{max}^{MeOH} 297 mμ (ε 5200); with nmr signals for two equatorial protons on hydroxyl-bearing carbons, at τ 4.90 (1H, br s, W_H = 5 Hz) and τ 5.39 (1H, br s, W_H = 8 Hz). Treatment of 4 with methanolic HCl for 60 min led quantitatively to a mixture of C-19-epimeric cycloacetals, 5a and 5b. Preparative tlc on alumina gave crystalline epimer 5a and amorphous epimer 5b, in the ratio 4:1. Crystalline epimer 5a, $C_{25}H_{34}O_7$, showed mp 246-248° d; $[\alpha]_D^{22}$ -23° (c 0.56, $CHCl_3$); M^+ m/e 446; and IR and nmr spectral properties supporting the assigned structure. Formation of the 3,19-cycloacetal 5a indicated that the C-3 hydroxyl group in 4 is β-oriented. Involvement of the C-3β-hydroxyl in the orthoacetate in bersaldegenin 1,3,5-orthoacetate requires that the C-1 and C-5 hydroxyl groups also be β-oriented. Assignment of β-configuration to the substituent at C-17 was based upon conversion of 1 to enol ether 2, $C_{27}H_{34}O_7$: mp 279-282 d; $[\alpha]_D^{22}$ -74° (c 2.28, $CHCl_3$); M^+ m/e 470; λ_{max}^{MeOH} 299 mμ; and IR and nmr spectral properties supporting the assigned structure. Enol ether 2 was formed upon treatment of 1 with 2% methanolic



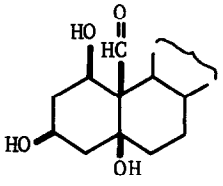
1



2



3



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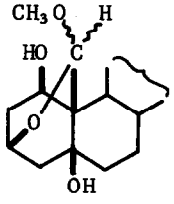
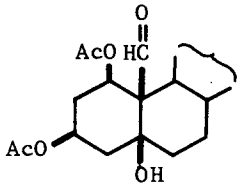
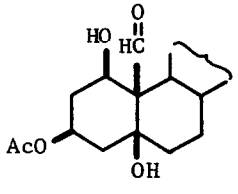


5a and 5b



6



7

NaOH for 2 hr at room temperature.

Bersaldegenin 1,3,5-orthoacetate (1) was synthesized from bersaldegenin (4) by a procedure described earlier for the synthesis of cyclohexane 1,3,5-triol orthoacetates.[9] Treatment of a suspension of 4 in ethyl orthoacetate-chloroform (1:1) with hydrogen chloride-saturated benzene for 4 days resulted in a 70% conversion to bersaldegenin 1,3,5-orthoacetate.

Bersaldegenin 3-acetate (7) was crystallized from methanol as rhombic prisms, $C_{26}H_{34}O_8$, mp 283-287° d; $[\alpha]_D^{22}$ -6° (c 1.19, $CHCl_3$-MeOH, 5:1); M^+ m/e 474; λ_{max}^{MeOH} 297 mμ (ε 5090); nmr signals at τ 4.55 (1H, br s, W_H = 9 Hz, C-3H), τ 5.00 (1H, br s, W_H = 5 Hz, C-1H), and τ 8.10 (3H, s, $OCOCH_3$). Mild alkaline hydrolysis of 7 gave 4; acetylation of 7 yielded 6. Chromatography of 3 or 7 on neutral alumina gave only unchanged starting material, indicative that 1 had not been formed from either 3 or 7 by alumina-catalyzed conversion during the isolation procedure. This observation and the results of thin layer chromatographic examination of the crude extracts support the view that bersaldegenin 1,3,5-orthoacetate (1) is, indeed, a naturally-occurring compound.

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