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The structure of velutinoside A: a pregnane pentasaccharide from *Mandevilla velutina*

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Abstract—Detailed spectroscopic analysis (IR, NMR and MS) has shown the structure of the potent bradykinin antagonist *velutinoside A* to be a pentasaccharide derivative of *velutinol A*, with the unusual sugars oleandrose and digitalose.

Mandevilla velutina is a native Brazilian plant used in folk medicine to treat snake bites and as an anti-inflammatory agent. Previous studies from this group have demonstrated that some pregnane compounds isolated from *Mandevilla velutina* and *Mandevilla illustris* were found effective in selectively antagonizing bradykinin and related kinin-mediated in vitro and in vivo responses.[1] Some of these compounds such as velutinol A[2] isolated from *M. velutina* and illustrol[3] isolated from *M. illustris* have been characterized chemically by our group. We report here the structure of a new compound,[4] a pentaglucoside, velutinoside A (**1**).

Compound **1**, a constituent (0.0001% of dry weight) of the rhizomes of *M. vellutina*, mp 148–150°C, white needles from $(C_2H_5)_2O$, responded positively to the Liebermann Burchard,[5] Xanthydrol[6] and Keller–Kiliane[7] reactions, indicating it to be a steroidal glycoside of a 2-deoxysugar. Elemental analysis gave C (60.26%), H (7.94%) and O (31.10%), and the hydrolysis (HCl) of velutinoside A gave velutinol A.[2] The IR spectrum (KBr) showed peaks at 3450 cm^{-1} (-OH), 1745 (-$COCH_3$), 2920, 1440 cm^{-1} (OCH_3); 1230, 1160, 1100, 1080, 1050 cm^{-1} (O-C-O). A methanolic solution is transparent in the UV-visible region. The positive ion FAB-MS afforded a molecular peak at m/z 1205 ($M+Na^+$); 1221 ($M+K^+$) and 1200 ($M+NH_4^+$), suggesting the molecular formula to be $C_{60}H_{94}O_{23}$. Major peaks in the FAB-MS were found at m/z (%) 1137 [M–CO–OH]$^+$ (66); 893 [1137-$C_{11}H_{16}O_6$]$^+$ (10); 749 [893-$C_7H_{12}O_3$]$^+$ (21); 605 [749-$C_7H_{12}O_3$]$^+$ (25); 462 [605-$C_7H_{11}O_3$]$^+$ (10); 318 [462-$C_7H_{12}O_3$]$^+$ (15). The peak at M–45 (m/z 1137) probably arises from the loss of the aglycone HC(15)O2 fragment, as observed in the MS of the parent velutinol A.[2] Subsequent loss of a fragment mass 244 (to give the peak with m/z 893) could be loss

Table 1. ^{13}C Chemical shift[a] assignments for the sugar rings in *velutinoside A*

	C1	C2	C3	C4	C5	C6	-OCH_3
R1	96.11	35.63	68.38	82.59	70.87	18.25*	56.74
R2	99.73	36.15	77.10	83.91	71.19	18.27*	58.33
R3	101.45	36.35	78.75	82.15	71.54	18.41	56.39
R4	98.47	35.37	76.39	83.81	69.29	18.06	58.03
R5	102.55	70.72	80.34	68.45	71.02	16.58	57.81

[a] Shifts in ppm to high frequency of TMS. (*) These assignments are interchangeable Ri = ring number (relative to the aglycone).

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of a terminal monodeoxy sugar with one methoxyl and two acetyl groups. Further sequential loss of fragments 144 (*m*/*z* 749), 144 (*m*/*z* 605), 143 (*m*/*z* 462) and 144 (*m*/*z* 318) suggests a chain of four more dideoxy sugars.

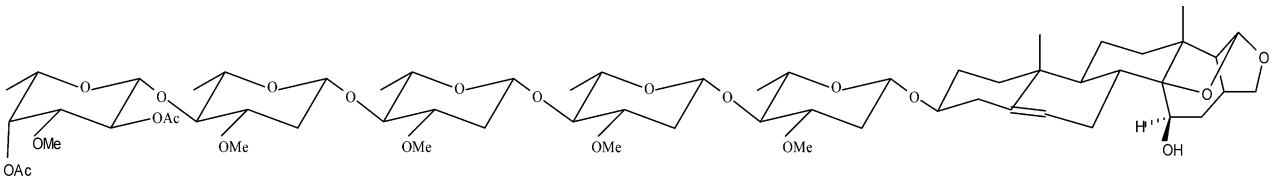
The strategy for the assignment of the ^{1}H and ^{13}C spectra started with the 150 MHz 1-D ^{13}C spectrum and related DEPT spectra. This gave a total of 55 resolved ^{13}C resonances grouped as 6 quaternary (2 carbonyls from their shifts), 26 methine, 10 methylene, and 13 methyl (4 of which are OCH_3 from their shifts, 56–58 δ). From the molecular formula ($C_{60}H_{94}O_{23}$) up to 5 of these resonances include accidental chemical shift equivalence, since signals at 35.5, 57.9, 68.3 and 82.5 δ were of greater intensity than the average of the others. The ^{13}C data for the sugar rings are collected in Table 1.

The 600 MHz ^{1}H NMR spectrum showed three distinct regions. The first (5.78–4.28 δ), with the best resolved signals, corresponds to the five anomeric protons and protons at C2 and C4 of the sugar ring 5, also protons 16, 6, 15(H), 15(OH), 20 and 21b of the aglycone (cf. velutinol A[2]). The second, crowded region (3.93–3.15 δ), integrated as 31 protons, assigned as protons 3 and 21a of the aglycone, five methoxy groups and fourteen methine protons of the sugar rings. Integration of the third region (2.53–1.08 δ) showed 50 protons; five secondary methyl groups, eight methylene protons, two acetyl methyl groups and 21 protons from the aglycone. Selective 1-D TOCSY experiments defined each of the five spin systems for the sugar rings attached to the aglycone. The four anomeric protons of the 2,6-dideoxyhexopyranose moieties were double doublets at 4.96; 4.85; 4.76 and 4.45 δ ($J=10$ and 2 Hz). A fifth anomeric proton appeared as a doublet ($J=10$ Hz) at 4.43 δ and was assigned to the normal hexapyranose unit. The large coupling constants of these anomeric protons were typical of the axial configuration of the hexopyranoses in the $^{4}C_{1}$(D) conformation indicating that these sugars were joined through (1→4) β-glycosidic linkages. The *J* coupling relationship described above was confirmed from the COSY spectrum. The spectrum also contained five methoxy singlets at 3.37 (3H), 3.44 (3H), 3.39 (3H), and 3.34 δ (6H); five secondary methyl doublets at 1.21 (9H), 1.22 (3H) and 1.29 δ (3H), ($J=6.0$ Hz); two tertiary methyl singlets at 1.08 and 1.11 δ and two acetyl methyls at 2.07 and 2.18 δ. The eight C-2 methylene protons of four 2-deoxy sugar units gave two sets of four-proton multiplets at 2.29–2.08 and 1.64–1.55 δ for the equatorial and axial protons respectively. A doublet of doublets at 5.12 δ ($J=10$ and 8 Hz) is due to the C2 proton of the acetylated sugar, coupled with both the signals at 4.43 δ (anomeric proton) and 3.33 δ (a C3 proton). The C3-H signal, part of a multiplet, is coupled with a double doublet at 5.33 δ ($J=3.0$ and 2.0 Hz) due to H4 of a diacetyl sugar, which in turn is coupled with a double doublet at 3.70 δ ($J=6.0$ and 2.0 Hz) due to the C5 proton. This is in turn coupled to a doublet ($J=6.0$ Hz) at 1.21 δ, due to the secondary methyl. The values for the coupling constants for H2 (10 and 8 Hz) and H4 (3.0 and 2.0 Hz) of the last sugar ring (5.12 and 5.33 δ, respectively) indicate the axial position at C2 and equatorial at C4. The ^{1}H data for the sugar rings are collected in Table 2. In addition, the HMBC spectrum correlated two ^{13}C carbonyls at 170.6 δ (acetyl at C2) and 172.4 δ (acetyl at C4), with methyls at 2.08 δ and 2.17 δ, respectively. The ^{1}H NOESY spectra also showed that in each of the sugar rings, the proton at C3 interacts with the proton at C1, and this is in turn spatially linked to a proton at C-5. These proximities can occur if the protons are all axial, and so provide evidence that the methoxy and the secondary methyl groups are located at equatorial positions. The evidence is that the sugars 1, 2, 3 and 4 are isostructural and are oleandrose. The

Table 2. ^{1}H Chemical shift assignments for the sugar rings in *velutinoside A*

	H1	H2		H3	H4	H5	H6	OCH_3
		ax	eq					
R1	4.85 dd (10;2)	1.58 (10;12;7)	2.08 (12;2;3.5)	3.81 (11;7;3.5)	3.22 dd (11;9)	3.85 dq (9;6)	1.22 d (6)	3.37
R2	4.76 dd (10;2)	1.64 (10;12;6)	2.11 (12;8;3)	3.78 (11;6;3)	3.19 dd (11;9)	3.34 dq (9;6)	1.21 d (6)	3.44
R3	4.45 dd (10;2)	1.58 (14;10;5)	2.29 (14;5;2)	3.40 (8.2;5)	3.15 dd (9;8.2)	3.28 (9;6)	1.29 d (6)	3.39
R4	4.96 dd (10;2)	1.55 (12;10;10)	2.16 (12;10;2.8)	3.77 (10;7;2.8)	3.19 dd (9;7)	3.92 dq (9;6)	1.21 d (6)	3.34
R5	4.43 d (10)	5.12 dd (ax) (10;8)		3.33 (10;3)	5.33 (3;2)	3.70 dq (6;2)	1.21 d (6)	3.34

The numbers in parentheses are the coupling constants in Hz; Ri = ring number (relative to the aglycone).



1

(15*R*,16*R*,20*S*)-3[(3-O-β-L-digitalosyl-(1→4)-O-β-L-oleandrosyl-(1→4)-O-β-L-oleandrosyl-(1→4)-O-β-L-oleandrosyl-(1→4)-O-β-L-oleandrosyl)oxy]-14,16:15,20:16,21-triepoxy-15,16-seco-14β,17α-pregn-5-en-15-ol

fifth sugar ring, a 2,4-diacetyl derivative is identified as digitalose. The NMR data confirm the sequence of the sugars indicated by FAB-MS with the 6-deoxy-2,4-acetoxy-3-*O*-methylhexopyranose at the terminus of the saccharide chain. The above evidence shows the structure of velutinoside A (**1**).

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References

1. (a) Calixto, J. B.; Nicolau, M.; Yunes, R. A. *Brit. J. Pharmacol.* **1985**, *85*, 729–731; (b) Calixto, J. B.; Nicolau, M.; Pizzolatti, M. G.; Yunes, R. A. *Brit. J. Pharmacol.* **1987**, *91*, 199–204; (c) Calixto, J. B.; Nicolau, M.; Yunes, R. A. *Gen. Pharmacol.* **1988**, *19*, 595–599; (d) Calixto, J. B.; Pizzolatti, M. G.; Yunes, R. A. *Brit. J. Pharmacol.* **1988**, *94*, 1133–1142; (e) Calixto, J. B.; Strobel, G. R.; Cruz, A. B.; Yunes, R. A. *Brazilian J. Med. Biol. Res.* **1988**, *21*, 1015–1018; (f) Calixto, J. B.; Yunes, R. A.; Rae, G. A.; Medeiros, Y. S. In *Nonpeptide Bradykinin Antagonists*: *Basic and Clinical Research*; Burch, R. M., Ed.; Marcel Dekker: New York, 1991; pp. 98–127; (g) Calixto, J. B.; Zanini, J. C., Jr.; Cruz, A. B.; Medeiros, Y. S. *Prostaglandins* **1991**, *41*, 515–526.
2. (a) Yunes, R. A.; Pizzolatti, M. G.; Sant'Ana, A. E. G.; Hawkes, G. E.; Calixto, J. B. *Phytochem. Anal.* **1993**, *4*, 76–81; (b) Bento, E. S.; Calixto, J. B.; Hawkes, G. E.; Sant'Ana, A. E. G.; Yunes, R. A. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1359–1366.
3. Yunes, R. A.; Brum, R. L.; Calixto, J. B.; Rimpler, H. *Phytochemistry* **1993**, *34*, 787–790.
4. Bkaily, G.; D'Orleans-Juste, P.; Calixto, J. B.; Yunes, R. A. *PCT Int. Appl.* WO 99 16,449, 8/4/99 (*Chem. Abs.* **1999**, *130*, 271976).
5. Abisch, E.; Reichstein, T. *Helv. Chim. Acta* **1960**, *43*, 1844–1861.
6. Barton, G. M.; Evans, R. S.; Gardner, J. A. F. *Nature* **1952**, *170*, 249–250.
7. Nagata, W.; Tamm, Ch.; Reichstein, T. *Helv. Chim. Acta* **1957**, *40*, 41–61.