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ROSEOSIDE–A C_{13} GLYCOSIDE FROM *VINCA ROSEA**

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Key Word Index—*Vinca rosea*; Apocynaceae; adenosine; roseoside; C_{13} glycoside structure and stereochemistry.

Abstract—Roseoside, a C_{13} glycoside isolated from *Vinca rosea* has been assigned the structure and stereochemistry (**1**).

INTRODUCTION

RECENTLY we reported the isolation and structure of a number of monoterpene glycosides of biogenetic interest from *Vinca rosea*.[1] Our continued interest in the chemistry of the water soluble fraction of this plant led to the isolation of adenosine and a new terpenoid glycoside named roseoside. The present communication describes essential data leading to the assignment of the structure and stereochemistry of this glycoside as **1** (R = β-D-glucose).

RESULTS AND DISCUSSION

Roseoside ($C_{19}H_{30}O_8$) was isolated as an amorphous powder after purification as the crystalline tetraacetate (**2**); subsequent deacetylation and silica gel column chromatography yielded the pure material. The UV spectrum λ_{max}^{MeOH} 237 and 316 nm (log ε 4·06 and 2·55 respectively) was characteristic of an enone system. The IR band at 1648 cm^{-1} supported this finding. The NMR spectrum showed the presence of two tertiary methyls τ 8·92 (*s*, 6H), a secondary methyl τ 8·62 (*d*, *J* 6·5 Hz), a methyl attached to an olefinic carbon τ 7·99 (*d*, *J* 1·5 Hz), an isolated methylene group α- to CO τ 7·52 and 3 olefinic protons at τ 4·05, 4·02 and 3·89 respectively.

Hydrolysis of roseoside with β-glucosidase yielded D-glucose and an aglycone (**3**). The presence of a–CO–CH=C–Me grouping in **3** was indicated by UV λ_{max}^{MeOH} 237 (log ε 4·05) and IR ν_{max}^{KBr} 1660 cm^{-1} and was confirmed by NMR signals at τ 8·09 (3H, *d*, *J* 1·5 Hz) together with a broad signal at τ 4·10 (1H). A strong band in the IR spectrum at 975 cm^{-1} and a 2H signal in the NMR spectrum at *ca* τ4·15 suggested the presence of a *trans*-disubstituted double bond. The signal at τ 8·72 (3H, *d*, *J* 6·5 Hz) and a broad multiplet at *ca* τ 5·60 (1H) showed the presence of a –CH(OH)–Me moiety. The NMR spectrum also had signals for two tertiary methyl groups at τ 8·98 and 8·92, a pair of isolated methylene protons centred at τ 7·67 and one olefinic proton at τ 4·20. These data suggest the formula **3** for the aglycone. When this work was completed we found the same structure proposed

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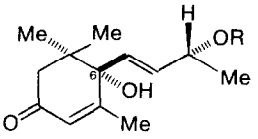
[1] BHAKUNI, D. S. and KAPIL, R. S. (1972) *Indian J. Chem.* **10**, 454.

for blumenol A, a compound isolated from the leaves of *Podocarpus blumei*[2] Endl. A similar gross structure had earlier been assigned to vomifoliol, an alcohol from *Rauwolfia vomitoria*[3] Afzel. Subsequently vomifoliol was also found to occur in *Croton sparsiflorus*[4] Morong.

Direct comparison of **3** and vomifoliol confirmed the identity. Blumenol A[5,6] was also found to be identical in all respects (m.p., m.m.p., TLC, superimposable IR and optical rotation) with **3**. The structure and stereochemistry of roseoside can, therefore, be represented as **1**.



(1) R = β-D-Glu $(OH)_4$
(2) R = β-D-Glu$(OAc)_4$
(3) R = H

Since, vomifoliol, blumenol A and B and theaspirone[7] have identical absolute configurations at C-6, it is probable that they are biosynthesized from (+)abscisic acid[8] by oxidative removal of the two terminal carbon atoms.

EXPERIMENTAL

Extraction. The fr. leaves and stems of *V. rosea* L. (11·5 kg) collected locally were percolated with 95% EtOH (5 × 10 l.). The solvent was removed *in vacuo* and the extract was concentrated to a small vol. (1 l.), diluted with H_2O (1 l.) and defatted with hexane (10 × 500 ml). The aq. portion was adjusted to pH 7 and extracted with $CHCl_3$–Et_2O (1:4) (6 × 250 ml) to remove basic material. The aq. portion was saturated with NaCl and re-extracted with $CHCl_3$–EtOH (4:1) (5 × 250 ml); this extract contained mainly polar alkaloids. The aq. portion was next extracted with *n*-BuOH (6 × 500 ml), the extract dried and concentrated under red. pres. to give an amorphous mass (8·2 g), which was found to be rich in glycosides. A portion of this material (4·7 g) was subjected to countercurrent distribution (10 ml, 100 transfers). The moving phase was *n*-BuOH-saturated with H_2O and the stationary phase was H_2O-saturated with *n*-BuOH.

Adenosine. Tube numbers 30–47 on concentration *in vacuo* afforded a residue (700 mg), which was chromatographed on a column of silica gel (60 g) and eluted with *n*-BuOH. Elution was continued with *n*-BuOH and 40 × 50 ml fractions were collected and examined by UV. Fractions 5–14 having a λ_{max}^{EtOH} 229 nm were combined and the solvent removed to afford a residue (400 mg). The NMR spectrum of this material showed it to be a mixture of sweroside, dehydrologanin and loganin. Similar work up of fractions 17–40 gave a crystalline residue, which was recrystallized from aq. MeOH to afford adenosine (10 mg); m.p. 228°; $(\alpha)_D$ − 58° (c, 1·0 H_2O); M^+ 267.

Roseoside (**1**). Tube numbers 66–73 after removal of solvent gave a residue (400 mg), which was chromatographed on a silica gel column (40 g) and eluted with $CHCl_3$ containing increasing proportions of MeOH. The eluate was monitored by NMR. It was found that although some of the fractions were quite homogenous, repeated attempts to crystallize the material from EtOH or other solvents were not successful and purification through the tetraacetate was used.

Roseoside tetraacetate (**2**). Roseoside (200 mg), Ac_2O (2 ml) and C_5H_5N (1 ml) were heated at 100° for 4 hr. Excess reagents were removed *in vacuo*, the residue was diluted with H_2O (10 ml) and extracted with C_6H_6 (3 × 10 ml). The C_6H_6 layer was washed with $NaHCO_3$ sol. H_2O, dried and the solvent removed to afford an amorphous material (220 mg). This material was purified by silica gel column chromatography (10 g) and elution with C_6H_6 to give (**2**), which was crystallized from EtOH (150 mg); m.p. 153°; $(\alpha)_D$ + 62° (c, 0·81, $CHCl_3$); λ_{max}^{MeOH}

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236 nm (log ε 4·08); ν_{max}^{KBr} 3395 (OH), 1755 (OAc), 1648 (enone) cm^{-1}; NMR ($CDCl_3$): τ 8·99 (*s*, 3H, CH_3CMe), 8·92 (*s*, 3H, $MeCCH_3$), 8·77 [*d*, *J* 6·5 Hz, 3H, $CH_3CH(OH)$], 8·10 (*d*, *J* 1·5 Hz, $CH_3C=$), 7·99 (*s*, 3H), 7·97 (*s*, 3H), 7·96 (*s*, 3H), 7·93 (*s*, 3H), 4 $CH_3O{=}CO$, 7·67 (*d*, 2H, $COCH_2$), 7·42 (*bh*, 1H), 6·34 (*bh*, 1H, $-CHOGlu$), 5·80 (*d*, 2H, CH_2OH), 5·49 (*m*, 2H), 4·99 (*m*, 3H), 4·05–4·20 (*m*, 3H, olefinic protons); M^+ 554 (Found: C, 58·60; H, 7·10. $C_{27}H_{38}O_{12}$ requires: C, 58·47; H, 6·90%).

Hydrolysis of roseoside tetraacetate (**2**). A soln of **2** (50 mg) in MeOH (10 ml) and $Ba(OH)_2$ (300 mg in 10 ml H_2O) was reacted at 20° for 20 hr. Excess MeOH was removed under red. pres. and the aq. soln was percolated through a column of freshly prepared Amberlite IR 120 resin (H^+) (6 g) to remove Ba^{2+}. The aq. soln on concentration afforded a residue (30 mg), which was further purified by chromatography on a short column of silica gel (2 g) and elution with EtOAc to afford pure roseoside (10 mg); $(\alpha)_D$ + 112° (c, 1·19 MeOH); λ_{max}^{MeOH} 237, 316 nm (log ε 4·06 and 2·55 respectively); ν_{max}^{KBr} 3390 (OH), 1648 (enone) cm^{-1}; NMR (D_2O): τ 8·92 (*s*, 6H, CH_3CCH_3), 8·62 (*d*, *J* 6·5 Hz, 3H, $CH_3CH(OH)$), 7·99 (*d*, *J* 1·5 Hz, 3H, $CH_3C=$), 7·52 (*d*, 2H, $CH_2O{=}C-$), 6·50 (*bs*, 3H), 4·05 (*d*, *J* 1·5 Hz, 1H), 4·02 (*s*, 1H), 3·89 (*bs*, 1H, $OCCH{=}C{-}Me$); (Found: C, 59·18; H, 7·94. $C_{19}H_{30}O_8$ requires: 59·05; H, 7·82%).

Enzymatic hydrolysis of roseoside (**1**). A soln of roseoside (10·6 mg) in H_2O (1·5 ml) was treated with β-glucosidase (25 mg) and two drops of toluene and the mixture kept at 35° under CO_2 for 24 hr. After cooling and dilution with EtOH (20 ml) the resulting ppt was filtered off and washed with EtOH. The combined EtOH extracts were concentrated *in vacuo* and the residue dissolved in H_2O (20 ml) and extracted with $CHCl_3$ (4 × 10 ml). The $CHCl_3$ layer was washed with H_2O, dried and the solvent removed to afford **3**, which crystallized as needles (4 mg) from C_6H_6-EtOAc m.p. 115°; $(\alpha)_D$ + 236° (c, 0·5 $CHCl_3$). λ_{max}^{MeOH} 237 nm (log ε 4·05); ν_{max}^{KBr} 3360 (OH), 1660 (enone) and 975 (*trans* disubstituted double bond) cm^{-1}; NMR ($CDCl_3$): τ 8·98 (*s*, 3H, CH_3CMe), 8·92 (*s*, 3H, $MeCCH_3$), 8·72 [*d*, *J* 6·5 Hz, $CH_3CH(OH)$], 8·09 (*d*, *J* 1·5 Hz, 3H, $CH_3C=$), 7·67 (*d*, 2H, methylene protons), 5·60 [*m*, 1H, $-CH(OH)Me$], 4·10–4·20 (*m*, 3H, olefinic protons), 6·72 and 6·93 (*bs*, 1H each, 2 OH, exchangeable with D_2O); M^+ 224 (Found: C, 69·45; H, 8·92. Calc. for $C_{13}H_{20}O_3$: C, 69·61; H, 8·99%).

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