

NERIASIDE, A 8,14-SECO-CARDENOLIDE IN NERIUM ODORUM

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(Received in Japan 8 March 1978; received in UK for publication 30 March 1978)

Many cardenolides have been isolated from the leaves, bark and seeds of Nerium odorum Sol. and N. oleander L. Previously we reported on the yellow pigments with 21-hydroxy-cardenolide framework in the root bark of N. odorum.<sup>1)</sup> In this communication we describe the isolation and structure elucidation of neriaside (I), a diginose of novel 8,14-seco-cardenolide in the leaves of N. odorum.

I ( $[\alpha]_D^{25} - 17.6^\circ$ ,  $\lambda_{\max}^{\text{MeOH}} 217\text{nm}$ ) was obtained as a minor constituent from the leaves dried at 80°, with the aid of polyamide as well as silica gel column chromatographies. The PMR of I indicates the presence of the butenolide ring by the characteristic resonances at 4.82 (2H, s, C-21) and 5.98 (1H, s, C-22), along with the peaks of 0.76, 0.80 (3H of each, s, C-18 and C-19), 1.34 (3H, d, J=6Hz, C-6'), 2.91 (1H, dd, J=9 and 9Hz, C-17), 3.44 (3H, s, C-3'-O-Me), 3.76 (1H, d, J=3Hz, C-4'), 4.15-4.35 (2H), and 4.58 (1H, dd, J=4 and 9Hz, C-1'). On acetylation with Ac<sub>2</sub>O and pyridine, I afforded a diacetate [II], solid, PMR: 0.78, 0.82 (C-18 and C-19), 1.20 (3H, d, C-6'), 2.15, 2.22 (3H of each, s, Ac), 2.97 (1H, dd, C-17), 3.36 (3H, s, C-3'-O-Me), 4.15 (1H, m, C-3), 4.57 (1H, dd, C-1'), 4.80 (2H, C-21), 5.01 (1H, d, J=5Hz), 5.19 (1H, d, J=3Hz, C-4'), 5.90 (1H, C-22)], in which no free hydroxyl was observed on IR.

I, on hydrolysis with 0.05N-H<sub>2</sub>SO<sub>4</sub>-50%MeOH for 1 hr, provided an aglycone (neriagenin)(III), mp 225-227°,  $[\alpha]_D^{25} - 1.6^\circ$ ,  $\lambda_{\max}^{\text{MeOH}} 215\text{nm}$ , C<sub>23</sub>H<sub>34</sub>O<sub>5</sub>: 390.2371) and diginose. On the PMR of III [0.76, 0.80 (C-18 and C-19), 2.89 (1H, dd, C-17), 4.12 (1H, d, J=4Hz), 4.28 (1H, m, C-3), 4.72 (2H, d, J=2Hz, C-21), 5.83 (1H, d, J=2Hz, C-22)]. The broad 1H multiplet at  $\delta$  4.28 is assignable to 3 $\alpha$ H by the comparison with that of digitoxigenin. The CMR of III reveals the presence of two carbinols (65.4ppm: C-3, and 79.5ppm), one carbonyl (216ppm) along with the butenolide carbons (172.6: C-20,

74.1: C-21, 116.8: C-22, 174.1: C-23) but no C-14 tertiary carbinol, which is usually observed at 85ppm. The A/B ring juncture is determined to be  $5\beta$  since C-18 methyl carbon is found at 17.7ppm.<sup>2)</sup> Taking into account of the molecular formula with the extra carbonyl, III seems unlikely as a normal cardenolide and a seco-cardenolide structure, cleaved on the bond between the carbinol and the carbonyl carbons, has been suggested.

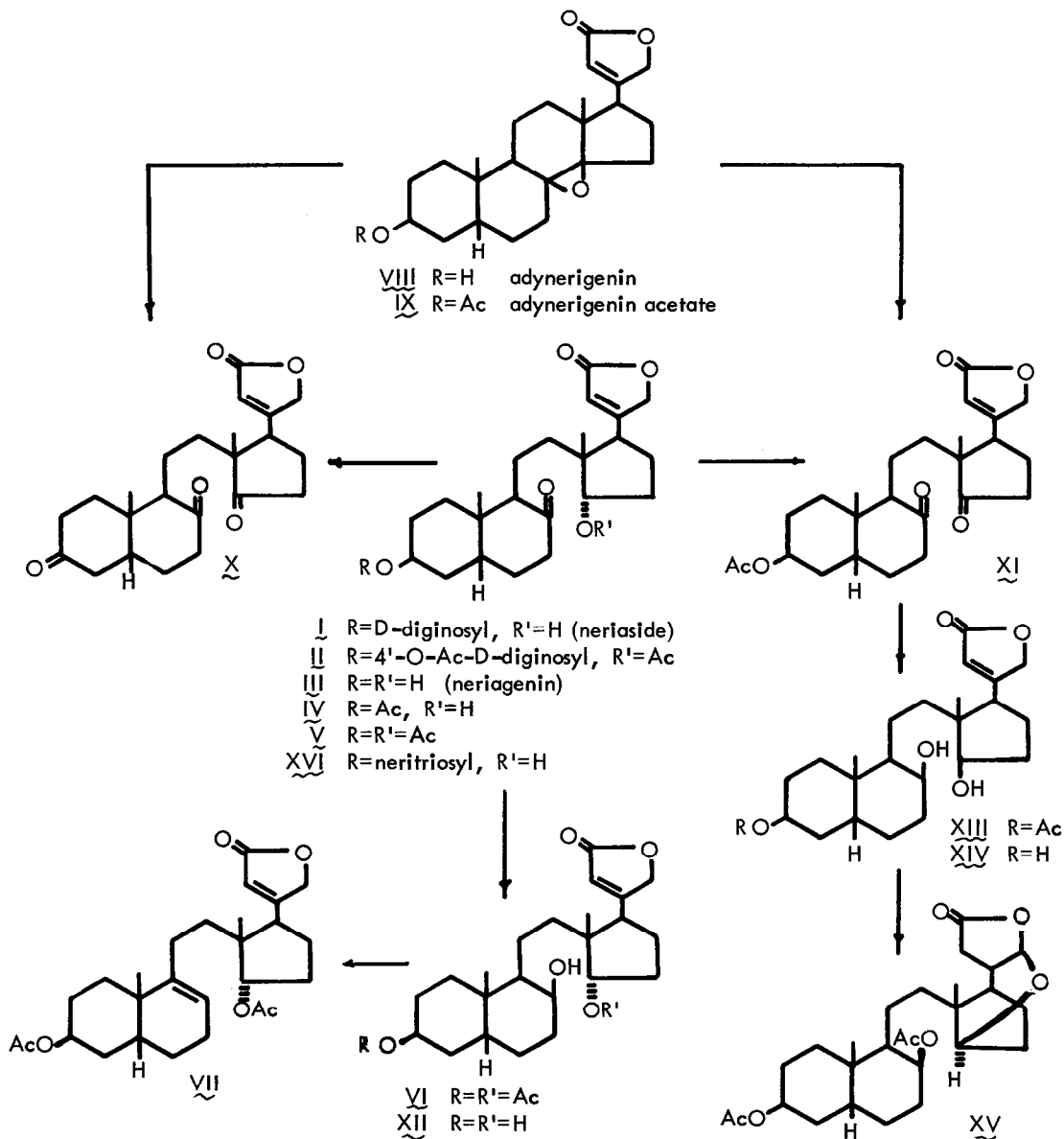
The second methine proton at  $\delta$  4.12 represents a similar coupling pattern as that of C-15 methine of some  $15\beta$ -hydroxy-cardenolides such as alloglaucotoxinin,<sup>3)</sup> and the location at D-ring is assumed. III, on heating with acetic acid at  $70^\circ$ , gave mono- and di-acetates (IV and V, respectively). IV [solid,  $C_{25}H_{36}O_6$ : m/e 433 ( $M^+ + 1$ )], is shown to be 3-O-acetate by downfield shift of the methine resonance from 4.28 to 5.18ppm. The remaining hydroxyl was acetylated with  $Ac_2O$ /pyridine to give V [mp  $197-198^\circ$ ,  $C_{27}H_{38}O_7$ : m/e 474.2604, PMR: 0.80, 0.82 (C-18 and C-19), 2.07, 2.22 (3H of each, Ac), 2.96 (1H, dd, C-17), 4.79 (2H, C-21), 5.03 (1H, d,  $J=5\text{Hz}$ ), 5.19 (1H, C-3), 5.90 (1H, C-22)], whereas it was unreactive with  $SOCl_2$  in pyridine. Thus, the second hydroxyl group has been assigned to C-14 in 8,14-seco-structure.

The carbonyl group in V was reduced with  $NaBH_4$  to give a triol diacetate [VI, mp  $165-168^\circ$ ,  $C_{27}H_{40}O_7$ : m/e 476.2757, PMR: 0.88, 0.92 (C-18 and C-19), 2.03, 2.06 (Ac), 2.96 (1H, C-17), 4.02 (1H, m), 4.80 (2H, C-21), 5.05 (2H, C-3 and C-14), 5.92 (1H, C-22)]. The PMR of VI exhibits upfield shift of  $3\alpha H$  by 0.14ppm comparing with V, possibly as a result of the reduction of the carbonyl. The newly formed methine proton is found at 4.02ppm in the similar pattern as that of  $3\alpha H$ , indicating the presence of an axial hydroxyl. Dehydration of VI with  $SOCl_2$  in pyridine provided an anhydride [VII, mp  $150-151^\circ$ ], in which only one olefinic proton was indicated at 5.31ppm as a broad multiplet. From these physical and chemical data, the location of the original carbonyl group has been defined at C-8.

In order to confirm the proposed structure of III, including the stereochemistry at C-17, the cleavage of 8,14-bond in the known cardenolide was examined. When adynerigenin (VIII) was treated with  $CrO_3$  in acetic acid, a trione (X, mp  $201-204^\circ$ ,  $\lambda_{\text{max}}^{\text{MeOH}}$  212nm,  $C_{23}H_{30}O_5$ : m/e 386) was obtained. The oxidation of III with  $CrO_3$  in pyridine also gave a trione, and the physical properties of the both triones were in good agreement. The similar reaction proceeded on both adynerigenin acetate (IX) and IV to give the identical dione [XI, mp  $188-189^\circ$ ,  $C_{25}H_{34}O_6$ : m/e 430.2353, PMR: 0.75, 0.78 (C-18 and

C-19), 2.04 (Ac), 3.36 (1H, C-17), 4.98 (2H, C-21), 5.20 (1H, C-3), 6.04 (1H, C-22)].

$\text{NaBH}_4$  reduction of III and XI afforded, respectively, a triol (XII, mp 145–147°) and a triol monoacetate [XIII, mp 98–103°,  $\text{C}_{25}\text{H}_{38}\text{O}_6$ : m/e 434, PMR: 0.80, 1.10 (C-18 and C-19), 2.04 (Ac), 2.71 (1H, C-17), 3.90–4.20 (2H, C-8 and C-14), 4.82 (2H, C-21), 5.00 (1H, C-3), 5.93 (1H, C-22)]. XIII was saponified to a triol (XIV, solid) which was distinguishable on TLC from XII. XIV, on treating with



0.3%KOH in MeOH, followed by acetylation, afforded a diacetate [XV, mp 223-227°,  $C_{27}H_{40}O_7$ : m/e 476, PMR: 1.05 (6H, C-18 and C-19), 2.03 (6H, Ac), 3.83 (1H, d, J=3Hz, C-14), 4.98-5.14 (2H, C-3 and C-8), 5.75 (1H, d, J=5Hz, C-21)], in which no hydroxyl is found on IR, and the formation of the "iso-linkage" between C-14 and C-21 is proved by the conversion of C-21 methylene protons at 4.82 into the methine at 5.75ppm as a same coupling constant as in isodigitoxigenin.<sup>4)</sup> Configuration of the hydroxyl at C-14 is determined as  $\beta$  in XIV, and consequently,  $\alpha$  in III. The structure of III has been elucidated as 3 $\beta$ , 14 $\alpha$ -dihydroxy-8, 14-seco-8-oxo-5 $\beta$ -card-20(22)-enolide.

Since only D-form of diginose has been known in Nerium, the sugar in I is regarded as D-diginose, the glycosidic linkage being assigned as  $\beta$  according to dd line of the anomeric proton at 4.58ppm, and also, the downfield shift of C-14 methine to 5.01ppm on the acetylation of I shows the attachment of D-diginose at C-3 hydroxyl.

A polar glycoside of I (XVI,  $[\alpha]_D^{20} -27.6^\circ$ ) was isolated as a solid from the air-dried leaves by means of the combination of silica gel and polyamide column chromatographies and a droplet counter-current chromatography. On the CMR of XVI, the resonances of the whole carbons belonging to III are represented, together with those arising from neritriose (=  $\beta$ -gentiobiosyl-D-diginose) moiety. The 8, 14-seco-cardenolide, therefore, appears to be a genuine substance in this plant.

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