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PREGNANES IN THE ROOT BARK OF *NERIUM ODORUM**

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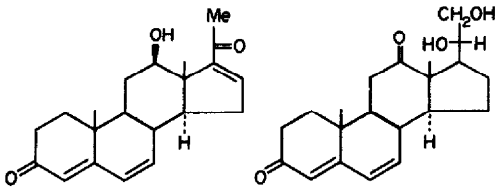
Key Word Index—*Nerium odorum*; Apocynaceae; oleander; pregnanes; defence substance.

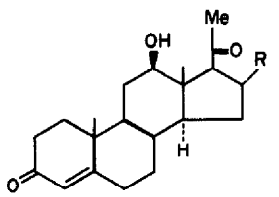
Abstract—Neridienone-A (12β-hydroxy-pregna-4,6,16-triene-3,20-dione), neridienone-B (20β,21-dihydroxy-pregna-4,6-diene-3,12-dione), 12β-hydroxy-pregna-4,6-diene-3,20-dione, 12β-hydroxy-pregn-4-ene-3,20-dione and 12β-hydroxy-16α-methoxy-pregna-4,6-diene-3,20-dione were obtained from the root bark of *Nerium odorum*.

INTRODUCTION

While many pregnane alkaloids have been isolated from Apocynaceae plants, only two genera in this family, *Holarrhena* and *Paravallaris*, are known to contain usual pregnanes [1–3]. During an investigation on the constituents of *Nerium odorum*, we previously reported pregnenolone glucosides [4]. This paper deals with the isolation and structure elucidation of the pregnanes from the same plant, including neridienone-A (**1**), neridienone-B (**2**).



1 Neridienone A 2 Neridienone B



3: R=H, Δ⁶
4: R=H
5: R=OMe, Δ⁶

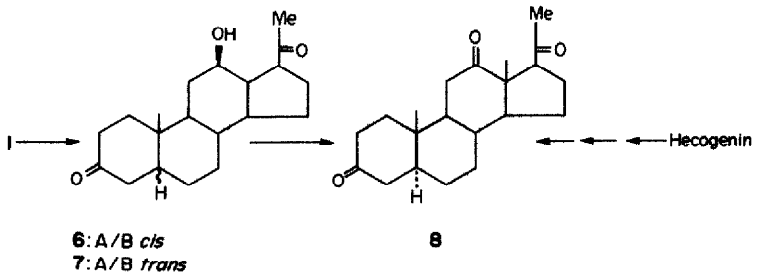
RESULTS

Several substances reacting positively to 2,4-dinitrophenylhydrazine reagent on TLC were observed in the benzene soluble compounds of a methanol extract of the root bark, and five of them (**1–5**) were isolated with the aid of column chromatography. According to colour reactions, molecular formulae, and NMR spectra, they were found to be pregnanes. Neridienone-A (**1**) was the major compound of the five and its structural elucidation has already been briefly described [5].

Neridienone-A (**1**) appeared to have 4,6-dien-3-one and 16-en-20-one systems, shown principally by absorptions in UV (283 and 245 nm) and IR (1665, 1640, 1612, 1580 cm^{-1}) spectra and by the presence of olefinic protons (δ 5.67, 6.12) revealed by NMR spectroscopy. The location and configuration of a hydroxyl group were suggested as 12β by double–doublet lines of a hydroxyl-methyne proton, coupled with adjacent methylene protons, centered at δ 3.71 with a spacing of 5 and 10 Hz, and by the fact that the dihydroderivative obtained by $NaBH_4$ reduction of **1**, afforded a diacetate whereas **1** was unreactive with Ac_2O and pyridine. The structure was finally confirmed by the direct comparison of the hexahydro-triketo derivative **8**, derived from **1** by CrO_3 oxidation following catalytic hydrogenation, with the authentic sample synthesized from hecogenin [6].

Neridienone-B (**2**), $C_{21}H_{28}O_4$, mp 179–181°, $[\alpha]_D$ +85.7°, isolated from the most polar fraction in the benzene extractives, also had the 4,6-dien-3-one system according to the absorption at 280 nm in the UV spectrum and the appearance of vinyl protons in the NMR spectrum. However, no acetyl methyl signal was observed in the region of δ 2.0–2.5, and instead, three protons, ascribable to hydroxyl-methynes were found at δ 3.4–3.9. On acetylation of **2** with Ac_2O/pyridine at room temperature, mono- and di-acetates (**9** and **10**) were obtained. The NMR spectrum of **9** indicated downfield shifts of two of the protons in **2** to δ 4.01 and 4.27, both as double-doublets, which were collapsed to doublets respectively by irradiation of the remaining multiplet at δ 3.55. These facts revealed that **2** contained a glycol system in which one hydroxyl was primary, and this was located at C-20, C-21. In addition to the 4,6-dien-3-one system, **2** was also considered to have another carbonyl group, whose location was assigned tentatively at C-12, on the basis of the C-12 oxygen function found in **1**, **3**, **4** and **5**. In order to elucidate the structure, **9** was converted into a saturated triketone (**13**) by Jones' oxidation

* Part 7 in the series "*Nerium*" by T. Y. For Part 6 see Yamauchi, T., Takahashi, M., and Abe, F. (1976) *Phytochemistry*, **15**, (in press).



of one of tetrahydro derivatives (**12**). On the other hand, an acetoxyl group was introduced at C-21 of 3β-hydroxy-5α-pregnan-12,20-dione upon reaction with $Pb(OAc)_4$ in benzene-methanol [7], and further oxidation of this product furnished a triketone, which was identical with **13**. As a result of the positive molecular rotation difference between the 20,21-di-*O*-acetate (**10**) and 20-hydroxy-21-*O*-acetate (**9**), the configuration of the hydroxyl at C-20 was assigned as β. The structure of **2**, therefore, was established as 20β,21-dihydroxy-pregna-4,6-diene-3,12-dione.

The crystals obtained from the column fraction eluted between **1** and **2** was found by UV and MS to be a mixture of two compounds, **3** and **4**, which were isolated as their acetates by PLC after acetylation. Compound **3**-acetate was shown by UV, NMR, and MS, to be a 4,6-diene-3-one containing an acetyl side chain at C-17, and one hydroxyl group. Therefore **3** was considered to be the 16,17-dihydro homologue of **1**, and the structure was proved by the catalytic hydrogenation of **3** to afford 12β-hydroxy-5β-, and-α-pregnane-3,20-dione (**6** and **7**), which had already been obtained from **1** by the same reaction conditions.

The structure of **4** was confirmed as 12β-hydroxy-progesterone by the UV absorption at 240 nm, absence of olefinic protons at C-6 and C-7 in the NMR spectrum, and by the similar MS patterns of **3**-acetate and **4**-acetate.

Compound **5**, $C_{22}H_{30}O_4$, was shown to have a 4,6-diene-3-one system, by UV absorption at 282 nm and NMR signals due to the olefinic protons. Additionally, the presence of a methoxyl group was shown by a signal at δ 3.39, which was eliminated to yield **1** in the acidic medium, accompanied by disappearance of 1 H multiplet at δ 4.20. Thus, **5** was a methanol adduct of **1**, the methoxyl residue being located at C-16. When **1** was treated with methanolic KOH, a methoxyl group was introduced into the 16-α position [8] and the product was identical with **5**. The configuration at C-16 was confirmed as α by a negative molecular rotation difference of **5**-acetate and **3**-acetate [6].

DISCUSSION

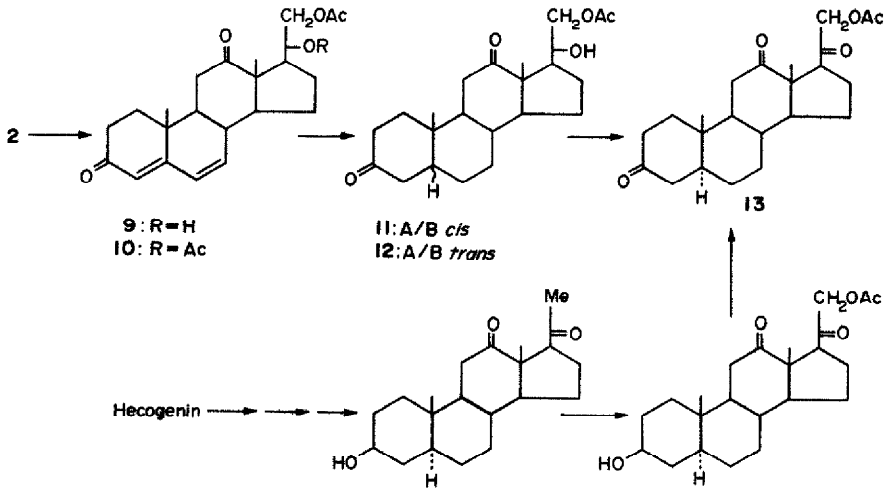
The 4,6-diene-3-one system, found in compounds **1**, **2**, **3** and **5** is present in the pregnane compounds obtained from *Paravallaris microphylla* [9]. and seems to be one of the characteristic features of the pregnanes isolated from Apocynaceae.

Although the formation of **5** was not observed when **1** was refluxed with methanol for 24 hr, the possibility of methoxylation during extraction or isolation can not be ruled out. While **5** is quickly converted into **1** with a trace amount of acid, it remains to be determined if it is **1** or **5** which is originally present in the plant.

Recently, compounds **3** and **4** were isolated from Indian water-beetles and they are known to act as defence substances against fish [10]. In the present examination neridienone-A and neridienone-B appeared to have similar toxic activities towards goldfish as those reported for cortexone and cybisterone derivatives [11].

EXPERIMENTAL

Extraction and isolation of pregnanes. Dried powdered root bark was percolated with MeOH. To the concentrated percolate, an equal vol. of H_2O was added and the mixture was



partitioned with hexane, C_6H_6, and then with $CHCl_3$. C_6H_6 extractives were subjected to Si gel column chromatography using C_6H_6–Me_2CO as eluant, and each fraction was checked on TLC with 2,4-dinitrophenylhydrazine reagent. Pregnanes, sensitive to the reagent, were isolated in the order: Compound **5** (0.0008%), neridienone-**A** (**1**) (0.037%), Compounds **3** and **4** (0.002%), and neridienone-**B** (**2**) (0.004%). **2** was also obtained from $CHCl_3$ extractives after Si gel column chromatography (0.001%).

Neridienone-A (**1**). Compound **1** was obtained as prisms on crystallization from hexane–EtOAc, mp 210–211°, $[\alpha]_D^{20}$ +71.5° (MeOH, *c* 0.21), (Found: C, 77.3; H, 8.1. $C_{21}H_{26}O_3$ requires: C, 77.3; H, 8.0%), MS: *m/e* 326.188. Calcd. 326.188; NMR (100 MHz, $CDCl_3$): δ 0.98 and 1.16 (3H each, *s*, C-18 and C-19), 2.43 (3H, *s*, C-21), 3.71 (1H, *dd*, J = 5, 10 Hz, C-12), 5.67 (1H, *d*, C-4), 5.81 (1H, *s*, C-12-OH), 6.12 (2H, *s*, C-6 and C-7), 6.99 (1H, *dd*, J = 2, 5 Hz, C-16); λ_{max}^{MeOH} nm(ε): 283 (24100), 245(12900); IR ν_{max}^{KBr} cm^{-1}: 3420, 3320, 1665, 1640, 1612, 1580.

$NaBH_4$ Reduction of **1**. Compound **1** (900 mg) was stirred with $NaBH_4$ (180 mg) in aq. EtOH at 0° for 1 hr. The mixture was diluted with H_2O, extracted with $CHCl_3$, and $CHCl_3$ extractives (850 mg) were chromatographed followed by acetylation to give two products [**1a** (200 mg) and **1b** (200 mg)]. **1a** (acetylated dihydroderivative of **1**): solid, λ_{max}^{EtOH} 282; MS: 412.233. $C_{25}H_{32}O_5$ requires 412.225; **1b** (acetylated tetrahydroderivative of **1**): mp 173–174°, MS: 414.240. $C_{25}H_{34}O_5$ requires 414.241; λ_{max}^{EtOH} 282.

Catalytic hydrogenation of **1**. Compound **1** (200 mg) was hydrogenated over Pd–C and two products [**6** (25 mg) and **7** (10 mg)] were obtained on crystallization from hexane–EtOAc following chromatography. **6**: mp 173–175°, MS: 332.233. $C_{21}H_{32}O_3$ requires 332.235; IR ν_{max}^{KBr} cm^{-1}: 3420, 1720, 1680. **7**: mp 157–160°, MS: 332.234. $C_{21}H_{32}O_3$; IR ν_{max}^{KBr} cm^{-1}: 3480, 1720, 1710.

Oxidation of **7**. Compound **7** was treated with Jones' reagent. The product was crystallized from Me_2CO to give needles (**8**), mp 208–212°, $[\alpha]_D^{20}$ +174° ($CHCl_3$, *c* 0.62); MS: 330.216. $C_{21}H_{30}O_3$ requires 330.220; IR ν_{max}^{KBr} cm^{-1}: 1710(br), 1670, which was identified as 5α-pregna-3,12,20-trione by direct comparison with authentic sample prepared from hecogenin via 3β-hydroxy-pregn-16-ene-12,20-dione (mmp, IR).

Neridienone **B** (**2**). On crystallization from hexane–Me_2CO, **2** was obtained as prisms, mp 179–181°. $[\alpha]_D^{20}$ +85.7° ($CHCl_3$, c 1.22), λ_{max}^{EtOH} nm (ε): 280 (32700); MS: 345 (M^+ + 1 H), (Found: C, 73.2; H, 8.3. $C_{21}H_{28}O_4$ requires: C, 73.2; H, 8.2%); NMR: δ 1.21 (6H, *s*, C-18 and C-19), 3.4 ~3.9 (4H, C-20, C-21 and −OH), 4.62 (1H, *bs*, −OH), 5.72 (1H, *s*, C-4), 6.09 and 6.20 (1H each, *d*, J = 10, C-6 and C-7); IR ν_{max}^{KBr} cm^{-1}: 3500, 3380, 1680, 1645, 1610, 1580. On acetylation, two acetates (**9** and **10**) were obtained. Compound **9**: mp 173–175°, $[\alpha]_D^{20}$ +59.8° ($CHCl_3$, *c* 0.64); MS: 387 (M^+ + 1 H); NMR: δ 1.18 (6H, *s*, C-18 and C-19), 2.08 (3H, *s*, −OCOMe), 3.55 (1H, *m*, C-20), 4.01 and 4.27 (1H each, *dd*, J = 5, 12, and 3,12, C-21), 4.46 (1H, *bs*, −OH), 5.71 (1H, *s*, C-4), 6.07 and 6.20 (1H each, *d*, J = 10, C-6 and C-7). Compound **10**: solid, $[\alpha]_D^{20}$ +78.6 ($CHCl_3$, *c* 3.44), $\Delta[M]_D = ([M]_D \cdot \mathbf{10} - [M]_D \cdot \mathbf{9}) = +106°$; MS: 428 ($M^+$); NMR: 1.14 and 1.18 (3H each, *s*, C-18 and C-19), 2.05 (6H, *s*, −OCOMe), 3.98 and 4.38 (1H each, *dd*, J = 7, 12, and 3, 12, C-21), 5.02 (1H, *m*, C-20), 5.74 (1H, *s*, C-4), 6.10 and 6.22 (1H each, *d*, J = 10, C-6 and C-7).

Catalytic hydrogenation of **9**. Compound **9** (200 mg) was shaken in EtOH with Pd–C for 2 hr. The products were fractionated on chromatography followed by crystallization from hexane–EtOAc to give **11** (30 mg) and **12** (40 mg), both as prisms. Compound **11**: mp 105–106°, MS: 390 (M^+); **12**: mp 193–203°, MS: 391 (M^+ + 1 H).

21-Acetoxy-5α-pregnan-3,12,20-trione (**13**). From **12**: **12** was treated with Jones' reagent to give prisms after crystallization from hexane–EtOAc; mp 222–223°, MS: 388.220. $C_{23}H_{32}O_5$ requires 388.225; NMR: δ 1.00 and 1.09 (3H each, *s*, C-18 and C-19), 2.13 (3H, *s*, −OCOMe), 4.70 and 5.12 (1H each, *d*, J = 21, C-21); IR ν_{max}^{KBr} cm^{-1}: 1750, 1722, 1703, 1240. (b) From hecogenin: 3β-Hydroxy-5α-pregnan-12,20-dione (270 mg), prepared from hecogenin, was dissolved in MeOH–C_6H_6 (1:9) containing BF_3 and stirred with 540 mg of $Pb(OAc)_4$ at room temp. for 12 hr, the mixture was washed with H_2O and the solvent was evaporated *in vacuo*. Residue (320 mg) was subjected to Jones' oxidation, followed by chromatography and crystallization from hexane–C_6H_6 to give 30 mg of prisms, mp 221–222°, identical with **13**, obtained from **12** in all respects.

12β-Hydroxy-pregna-4,6-diene-3,20-dione (**3**) *and 12β-hydroxy-pregn-4-en-3,20-dione* (**4**). The mixture of **3** and **4** (mp 150–155°) was acetylated and subjected to PLC with hexane–Me_2CO (3:1) as developing solvent and **3**-acetate and **4**-acetate were isolated. **3**-acetate: mp 153–154°, $[\alpha]_D^{20}$ +89.0° ($CHCl_3$, *c*, 0.87); λ_{max}^{MeOH} nm(ε): 283 (26200); MS: 370.215 (M^+), 310, 268. $C_{23}H_{30}O_4$ requires 370.214; NMR: δ 0.94 and 1.11 (3H each, *s*, C-18 and C-19), 2.01 and 2.08 (3H each, *s*, $-COCH_3$), 4.80 (1H, *dd*, J = 5, 11, C-12), 5.71 (1H, *s*, C-4), 6.06 and 6.18 (1H each, *d*, J = 11, C-6 and C-7). Tetrahydroderivative of **3**: compound **3** (160 mg) was treated with Pd–C in EtOH and the products [**6** (30 mg) and **7** (20 mg)] were obtained after chromatography; **6**, mp 170–175°, MS: 332.236. $C_{21}H_{32}O_3$ requires 332.235; IR ν_{max}^{KBr} cm^{-1}: 3420, 1720, 1680; was identical with authentic material on direct comparisons (mmp, IR). **7**, mp 157–159°, MS: 332.226. $C_{21}H_{32}O_3$; IR ν_{max}^{KBr} cm^{-1}: 3480, 1720, 1710; was identical with an authentic sample. **4**-acetate: solid, λ_{max}^{EtOH} nm: 240; MS: 373.234 (M^+ + 1H), 312, 270. $C_{23}H_{32}O_4$ + 1H requires 373.238; NMR: δ 0.88 and 1.18 (3H each, *s*, C-18 and C-19), 2.00 and 2.05 (3H each, *s*, −COMe), 4.75 (1H, *dd*, J = 5, 11, C-12), 5.75 (1H, *s*, C-4).

12β-Hydroxy-16α-methoxy-pregna-4,6-diene-3,20-dione (**5**). Compound. **5** was crystallized from hexane–C_6H_6, mp 168–171°, λ_{max}^{MeOH} nm(ε): 282 (16100); MS: 358.210. $C_{22}H_{30}O_4$ requires 358.214; NMR: δ 0.80 and 1.10 (3H each, *s*, C-18 and C-19), 2.23 (3H, *s*, C-21), 3.40 (3H, *s*, −OMe), 3.61 (1H, *dd*, J = 5, 10, C-12), 4.20 (1H, *m*, C-16), 4.65 (1H, *bs*, −OH), 5.68 (1H, *s*, C-4), 6.06 and 6.20 (1H each, *d*, J = 11, C-6 and C-7). **5**-acetate: solid, $[\alpha]_D^{20}$ + 7.7° (MeOH, *c* 0.90). $\Delta(M)_D = ([M]_D \cdot$ **5**-acetate $- [M]_D \cdot$ **3**-acetate) = −298°. Compound **5** (20 mg) in $CHCl_3$ was allowed to stand overnight with a small amount of trifluoroacetic acid. Solvent was evaporated *in vacuo* and the residue was crystallized from hexane–EtOAc to yield **1** (mp 205–206°, mmp, IR).

Compound **5** *from Neridienone-A*. **1** (500 mg) was refluxed with methanolic KOH (0.6 g/20 ml) for 30 min, and the reaction mixture was diluted with H_2O. The product was extracted with $CHCl_3$, followed by crystallization from hexane–C_6H_6 to give needles, mp 167–169° and identified as **5** on direct comparisons (mmp, IR). When **1** was dissolved in MeOH and refluxed for 24 hr, only **1** was detected on TLC.

Activity of the pregnanes against goldfish. **1** and **2** were ground with Tween 20, diluted with H_2O and the concentration of the pregnanes was adjusted to 5 mg/l respectively. Goldfishes (5 fishes each) were placed in the pregnane soln. The solns of **1** and **2** required 8 and 13 min, respectively, until fishes went down to the bottom of vessel and ceased to swim, while no change on fishes was observed in the solution of cholesterol at the concentration of 20 mg/l.

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