240° (lit. mp 225–26° and 232–33°), λ_{max} 226 nm (log ε 3.9) yielded a monoacetate, mp 224°, λ_{max} 225 nm (log ε 3.8). It was identified as 3β-hydroxylup-20(30)-en-29-al (**1**) by its co-chromatography, superimposable IR and mmp, and those of its acetate with the authentic samples. The authentic sample of **1**-acetate was obtained by SeO_2 oxidation of lupeol acetate [3]. The alkaline hydrolysis of the latter yielded **1**.

Since 3β-hydroxylup-20(30)-en-29-al was formed by allylic oxidation of wallichenol, the structure of wallichenol was established as lupan-20(30)-en-3β,29-diol (**2**)†. This assignment was further confirmed by the catalytic hydrogenation of the mixture of wallichianol and wallichenol which yielded a completely homogeneous product identical with wallichianol in all respects.

EXPERIMENTAL

All mps are uncorr. The ^1H NMR spectra were recorded at 60 MHz in $CDCl_3$ unless otherwise stated. A 3% OV-1 column was used for GC at 262° and He was the carrier gas. R_f values refer to TLC on Si gel plates in C_6H_6–MeOH (96:4).

MnO_2 oxidation of wallichianol–wallichenol mixture. The mixture of wallichianol and wallichenol (100 mg) was dissolved in $CHCl_4$ (10 ml) and stirred with MnO_2 (100 mg) for 24 hr, then filtered. The filtrate was evapd. The product showed 2 spots of R_f 0.47 and 0.68 on TLC. The product was chromatographed over Si gel. The $CHCl_3$ eluate yielded the product of higher R_f (**1**, 45 mg), mp 240°, λ_{max} 225 nm (log ε 3.9). (Found: C, 81.9; H, 10.65. $C_{30}H_{48}O_2$ requires: C, 81.9; H, 10.9%).

On acetylation with C_6H_5N–Ac_2O **1** yielded an acetate mp 224°, λ_{max} 225 nm (log ε 3.8).

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† Lupan-20(30)-en-3β,29-diol obtained by reduction of 3β-acetoxylup-20(30)-en-29-al has a mp 231–2° [4].

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PREGNANES OF *ANODENDRON AFFINE*

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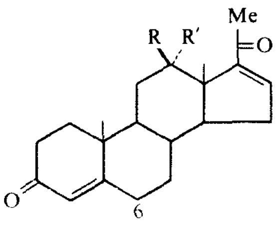
Anodendron affine Druce is indigenous to the southern part of Japan. Previously, the constituents of the trunk were investigated by Inagaki *et al.* [1] and pyrrolizidine alkaloids were isolated from the leaves by Sasaki and Hirata [2]. Cardenolides bearing a 4,6-dideoxyhexosone were isolated from the same genus, and the structures were determined by Lichti *et al.* [3]. We now report the identification of four pregnanes with 4,16-dien-3-one and 4,6,16-trien-3-one functions.

Four compounds (**1**–**4**) were obtained as crystals from the MeOH percolate of the trunk with bark by partitioning the MeOH extractives with benzene followed by silica gel chromatography.



1 R = OH, R' = H, Δ^6
2 R = OH, R' = H
3 R,R' = =O, Δ^6
4 R,R' = =O

1 was identified as neridienone A, a pregnane derivative previously isolated from the root bark of *Nerium indicum* [4], by direct comparison with an authentic sample. **2** shows a lower M^+ than **1** by 2 mass units, and neither an absorption maximum at 284 nm nor the 2H resonance at δ 6.13, due to the C-6 and C-7 olefinic protons in a 4,6-dien-3-one system. Since an absorption due to the Δ^{16}-20-one was observed at 245 nm, and a C-16 olefinic proton at δ 7.00 as in **1** in lower field than a 12-deoxy derivative, the structure of **2** was determined as 12β-hydroxy-4,16-pregnadien-3-one.

The results of MS and ^{1}H NMR analyses showed a third fraction to be a mixture of a 4,6-dien-3-one and a 4-en-3-one (**3** and **4**). In order to verify the structures of **3** and **4**, the mixture was subjected to catalytic hydrogenation to give two compounds, one of which was identified as 12β-hydroxy-5α-pregna-3,20-diene and the other, as the 5β-isomer, by the comparison with the authentic samples prepared from **1**. The oxidation of **1** with CrO_3/pyridine afforded a small amount of a trione, in which the peaks in MS and ^{1}H NMR were identical to those of **3**. The structure of **3** was therefore elucidated as 4,6,16-pregnatriene-3,12,20-trione and **4** as 4,16-diene-3,12,20-trione.

As already described [4], **1** shows piscicidal activity, arresting the movement of goldfish within 8 min in 5 ppm solution or 3 min in 10 ppm. **2** and **3** also show activity of 3 min/20 ppm and 8 min/20 ppm, respectively.

EXPERIMENTAL

Plant material. Plants were collected at Madarajima of Saga Pref., Japan in March, 1975 and identified by Mr. T. Tateishi of Faculty of Sciences, Fukuoka University (voucher specimen No. 54-01; Herbarium of the Experimental Station of Medicinal Plant Studies, Kyushu University).

Extraction and isolation of pregnanes. Dried powdered trunk with bark (24 kg) was percolated with MeOH, and the MeOH was evapd *in vacuo*. The concentrate was diluted with an equal vol. of H_2O, extracted with hexane (yielding 8.8 g), and then with C_6H_6 (yielding 32 g). The C_6H_6 extractives were subjected to Si gel chromatography with C_6H_6–Me_2CO as eluent to give a fraction which showed positive reactions with Kedde (blue) and 2,4-dinitrophenylhydrazine reagents, prior to the fractions containing cardenolides. This material was further chromatographed with hexane–EtOAc, yielding 3 fractions; each fraction, crystallized from EtOAc–hexane, gave needles (fraction 1: 0003 %, 2: 0.0007 %, 3: 0.0002 %).

Fraction 1 (**1** = *neridienone* A). mp 208°; $[\alpha]_D^{25}$ +76.5° (MeOH, *c* 0.11); λ_{max}^{MeOH} nm (ε): 245 (12000), 284 (23000). ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.96 and 1.13 (3H of each, *s*, C-18 and C-19), 2.40 (3H, *s*, C-21), 3.69 (1H, *dd*, J = 5, 10 Hz, C-12), 5.70 (1H, *s*, C-4), 5.79 (1H, *s*, C-12-OH), 6.13 (2H, *s*, C-6 and C-7), 7.00 (1H, *dd*, J = 2, 5 Hz, C-16). MS (probe) 70 eV *m/e*: 326 $[M^+]$. Physical properties in good agreement with those of authentic neridienone A.

Fraction 2 (**2**). mp 235°; $[\alpha]_D^{25}$ +100° (MeOH, *c* 0.11); λ_{max}^{MeOH} nm (ε): 240 (26000): ^{1}H NMR: δ 0.92 and 1.22 (3H of each, *s*, C-18 and C-19), 2.39 (3H, *s*, C-21), 3.71 (1H, *dd*, J = 5, 10 Hz, C-12), 5.75 (1H, *s*, C-4), 5.83 (1H, *s*, C-12-OH), 7.00 (1H, *dd*, J = 2, 5 Hz, C-16). MS: 328 $[M^+]$.

Fraction 3. mp 232°; λ_{max}^{MeOH} nm (ε): 230 (11900), 280 (8300). ^{1}H NMR: 1.22. 1.42, 2.32, 5.73, 6.20, 6.64 (for **3**), 1.30, 1.38, 2.39, 5.78, 6.64 (for **4**). MS: 324, 326 $[M^+]$, 309, 311 $[M^+ - 15]$, 296, 298 $[M^+ - 28]$, 281, 283 $[M^+ - 43]$. **1** (300 mg) in 10 ml of Py was stirred with CrO_3/Py (1 g in 20 ml) at room temp. for 3 days. The reaction mixture was chromatographed on a Si gel column and crystallized from hexane–EtOAc to give 10 mg of prisms, mp 228–235°. ^{1}H NMR: δ 1.22 and 1.41 (3H of each, C-18 and C-19), 2.33 (3H, *s*, C-21), 5.73 (1H, *s*, C-4), 6.18 (2H, *s*, C-6 and C-7), 6.64 (1H, *dd*, J = 2, 2 Hz, C-16). MS: 324 $[M^+]$. Fraction 3 (67 mg) was shaken with PtO_2 (30 mg) in an atmosphere of H_2 for 50 min, and the resultant products were examined by GLC, RT: 8.7 and 9.8 min (specimen 5β- and 5α-pregnane-3,12-20-trione: 8.7 and 9.8 min, respectively, with 2.1 m SE-52 column at 228°, carrier: N_2, 1.0 atm). The products were isolated by chromatography on a Si gel column. The IR of product 1, mp 206–208° and of product 2, mp 204–206°, were in good agreement with those of 5β- and 5α-pregnane-3, 12, 60-trione [4], respectively, prepared from **1**.

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