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# PREGNENOLONE GLUCOSIDES OF *NERIUM ODORUM**

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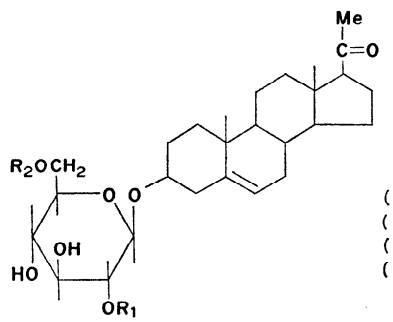
**Key Word Index**—*Nerium odorum*; Apocynaceae; pregnenolone glucosides.

**Abstract**—The β-D-glucopyranoside, β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside, β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside and *bis*-β-D-glucopyranosyl-(1 → 2, 1 → 6)-β-D-glucopyranoside of pregnenolone were obtained from root and trunk bark of *Nerium odorum*.

WHILE characteristic pregnanes are known in apocynaceous plants,[1] there are no reports on *Nerium oleander* or *N. odorum*, species containing cardiac glycosides, except for the description of dienone compound in the seeds of *N. oleander* by Jäger *et al.*[2] We now describe the isolation and the structure elucidation of pregnenolone ($\Delta^5$-pregnen-3β-ol-20-one) glucosides of *N. odorum* Sol.




(I) $R_1 = R_2 =$ Glc
(II) $R_1 =$ H ; $R_2 =$ Glc
(III) $R_2 =$ H ; $R_1 =$ Glc
(IV) $R_1 = R_2 =$ H

Four substances reacting positively to the Carr–Price test were obtained from the root and trunk bark along with the cardiac glycosides, following solvent extraction and column chromatography, and these were labelled I–IV in order of decreasing polarity.

On complete acid hydrolysis, they gave, besides pregnenolone, 3 mol of glucose from I, 2 from II and III and 1 from IV, respectively. On partial hydrolysis, I furnished II, III, IV, pregnenolone, glucose and gentiobiose, while both II and III afforded IV and pregnenolone. Gentiobiose was also obtained on partial hydrolysis of II as well as of I. IV, therefore, was pregnenolone monoglucoside and I appeared to be triglucoside, with a branched chain sugar. Permethylates of I, II and III, prepared by the Kuhn method,[3] gave, on methanolysis, methyl 3,4-di-*O*-methylglucoside, methyl 2,3,4-tri-*O*-methylglucoside and methyl 3,4,6-tri-*O*-methylglucoside, respectively, together with methyl 2,3,4,6-tetra-*O*-methylglucoside.

* Part II in the series "*Nerium*". For Part I see T. YAMAUCHI and Y. EHARA, *Yakugaku Zasshi* **92**, 155 (1972).

[1] R. TSCHESCHE, *Angew. Chem.* **73**, 727 (1961); *Fortsch. Chem. Org. Naturstoffe*, Vol. 24, p. 111, Springer, Berlin (1966).
[2] H. JÄGER, O. SCHINDLER and T. REICHSTEIN, *Helv. Chim. Acta* **42**, 977 (1959).
[3] R. KUHN, I. LÖW and H. TRISCHMANN, *Chem. Ber.* **88**, 1492, 1690 (1955).

From the comparison of molecular rotations of the glucosides and of coupling constants of anomeric protons of the permethylates (Tables 1 and 2), and from the good agreement in physical properties of IV with that of synthetic pregnenolone β-D-glucopyranoside, the linkage between glucose and glucose, and glucose and aglycone in the glucosides were all deduced to be β-form. Hence, the structures of I, II, III and IV were verified as $\Delta^5$-pregnen-3β-ol-20-one *bis*-β-D-glucopyranosyl-(1→2, 1→6)-β-D-glucopyranoside, β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, β-D-glucopyranosyl-(1→2)-β-D-glucopuranoside and β-D-glucopyranoside, respectively.

TABLE 1. MOLECULAR ROTATIONS OF GLUCOSIDES

| $[M]_D$ | Differences of $[M]_D$ | | Linkage |
|---|---|---|---|
| II : −76° | II–IV | −13° | glc.-(1→6)-glc. |
| III : −46° | III–IV | +17° | glc.-(1→2)-glc. |
| IV : −63° | IV–pregn. | +196° | glc.-pregn. |
| Pregnenolone : +133° | | | |
| Me α-D-glucopyranoside +307° | | | |
| Me β-D-glucopyranoside −63° | | | |

TABLE 2. ANOMERIC PROTONS OF GLUCOSIDES PERMETHYLATES

| | Chemical shift (ppm) | Coupling constants (Hz) |
|---|---|---|
| IV-Permethylate | 4·34(d) | 7 |
| III-Permethylate | 4·05(d), 4·30(d) | 9, 7 |
| II-Permethylate | 4·42(d), 4·65(d) | 7, 7 |

Pregnenolone is known as an biosynthetic precursor of cardenolides, but it has only been isolated from two species, *Xysmalobium undulatum*[4] and *Trachycalymna fimbriatum*,[5] in the former as a mixture with pregnan-3β-ol-20-one. It is noteworthy that pregnenolone glucosides are obtained in good yield and in a pure state from *N. odorum*.

## EXPERIMENTAL

GLC was taken with 5%, 1,4-butanediol succinate column at 170° of column temp. TLC was developed on a Kiesel gel G nach Stahl, with $CHCl_3$-MeOH-$H_2O$ (7:3:1, lower), or hexane-EtOAc (1:3). PC for sugars was run with (a) BuOH-pyridine-$H_2O$ (6:2:3, upper) + pyridine (1) or (b) EtOAc-pyridine-$H_2O$ (2:1:2, upper).

*Extraction and isolation of pregnenolone glucosides* (I–IV). Dried powdered root bark of *Nerium odorum* (1·8 kg) was extracted with $Et_2O$ (ext. 117 g) and defatted bark was percolated with MeOH. MeOH solution was concentrated *in vacuo* to *ca.* 1 l., diluted with $H_2O$, and the solution was extracted with $CHCl_3$ (ext. 64 g) and then with BuOH (ext. 83 g). BuOH and $CHCl_3$ exts. were chromatographed on silica gel with $CHCl_3$–MeOH–$H_2O$ (7:3:1, lower) and with $CHCl_3$–MeOH, respectively. The corresponding fractions were rechromatographed and crystallized from EtOH or MeOH–$H_2O$ to give 8·5 g (yield 0·47%) of I, 4·5 g (0·24%) of II and 1·6 g (0·09%) of III. From $CHCl_3$ ext., 6 mg of IV was obtained. Powdered trunk bark (1·4 kg) was treated in the foregoing procedure and I (1·7 g), II (0·71 g) and III (0·44 g) were obtained.

*Physical constants of the glucosides.* I: m.p. 231–235° dec., $[\alpha]_D^{20}$ −20·5° in pyridine (*c*, 0·4) (Found: C, 57·1, H, 8·0. $C_{39}H_{62}O_{17}.H_2O$ requires: C, 57·1; H, 7·9%). I Acetate: m.p. 185–187°, $[\alpha]_D^{20}$ +6·4° in $CHCl_3$ (*c*, 1·5). I Permethylate was not crystallized. II: m.p. 255–259° dec., $[\alpha]_D^{20}$ −11·9° in pyridine (*c*, 0·7) (Found: C, 61·9; H, 8·3. $C_{33}H_{52}O_{12}$ requires: C, 61·9; H, 8·2%). II Acetate: m.p. 190–194°, $[\alpha]_D^{20}$ +1·2° in $CHCl_3$ (*c*, 1·7) (Found: C, 59·8; H, 7·2. $C_{47}H_{66}O_{19}$ requires: C, 60·4; H, 7·1%). II Permethylate: m.p. 71–75°, $[\alpha]_D^{20}$ +24·1° in $CHCl_3$ (*c*, 0·8). III: m.p. 252–256° dec., $[\alpha]_D^{20}$ −7·2° in pyridine (*c*, 0·7) (Found: C, 59·0; H, 8·3. $C_{33}H_{52}O_{12}.2H_2O$ requires: C, 58·6; H, 8·3%). III Acetate: m.p. 160–166°, $[\alpha]_D^{20}$ +8·6° in $CHCl_3$ (*c*, 0·6) (Found: C, 60·1; H, 7·2. $C_{47}H_{66}O_{19}$ requires: C, 60·4; H, 7·1%), III Permethylate: m.p. 105–107°, $[\alpha]_D^{20}$ −16·0° in $CHCl_3$ (*c*, 1·6). IV: m.p. 269–271° dec. IV Permethylate: m.p. 124–126°, $[\alpha]_D^{20}$ +10·8° in $CHCl_3$ (*c*, 0·7). I–IV gave pink colors on TLC plate after spraying with $SbCl_3$ in $CHCl_3$ (Carr–Price reagent), followed by heating in the oven.

*Hydrolysis.* (a) I, II, III or IV (10 mg each) was refluxed with 1 ml of 2 N HCl–30% EtOH for 1 hr and aglycone and sugar portions were separated in usual manner. On TLC, aglycone portion of I–IV gave one main spot coincided with pregnenolone and minor spot, probably assigned as pregna-3,5-diene. On PC, sugar portions of I, II, III and IV indicated glucose only. (b) II (300 mg) was refluxed with 30 ml of 2 N

[4] R. TSCHESCHE and G. SNATZKE, *Ann.* **636**, 105 (1960).

[5] R. ELBER, Dissertation, Basel (1964); from J. VON EUW and T. REICHSTEIN, *Helv. Chim. Acta* **47**, 711 (1964).

HCl–50% EtOH for 2 hr, and EtOH was evaporated *in vacuo*. The ppt. was purified on silica gel column, followed by crystallization from dil. EtOH to give 90 mg of prisms, m.p. 192–195°, $[\alpha]_D^{20}$ +32·0° in pyridine (*c*, 0·5), and was in good agreement on direct comparison with authentic sample of pregnenolone (m.m.p., TLC, IR, ORD).

*Quantitative estimation of glucose.* Known amounts of I, II, III and IV were hydrolyzed with 1 N HCl–30% EtOH for 2·5 hr, EtOH was evaporated *in vacuo* and the mixture was filtered. The volume of the filtrate was adjusted to 50 ml with $H_2O$ and the aliquate was subjected to estimation of glucose with anthrone reagent.[6] Yield of sugar from the glucosides: Found: I, 65·9, 67·8; II, 59·1, 57·7; III, 60·2, 60·7; IV, 36·7, 37·1% (calc. for triglucoside: 67·9; diglucoside: 56·2; monoglucoside: 37·6%).

*Partial hydrolysis.* I (10 mg) was refluxed with 1 ml of 0·5 N HCl–30% EtOH for 1·5 hr and worked up in the usual manner. On TLC, 4 spots, coincided with II, III, IV and pregnenolone were observed and glucose ($R_{glc}$ 1·00) and gentiobiose (0·48) were detected on PC (specimen glucose 1·00, gentiobiose 0·49, Solv. B). II (10 mg) was refluxed with 1 ml of 0·2 N HCl–30% EtOH for 3 hr. On TLC, two spots were coincided with pregnenolone and IV. III (10 mg) was refluxed with 1 ml of 0·5 N HCl–50% EtOH for 1 hr. On TLC, two spots were coincided with pregnenolone and IV respectively.

*Preparation of* IV. II (600 mg) was refluxed with 60 ml of 0·2 N HCl–30% EtOH for 3 hr and EtOH was evaporated *in vacuo*. Precipitate, obtained by filtration, was purified on column chromatography, followed by crystallization from MeOH–$H_2O$ to give 86 mg of needles, m.p. 270–271° dec., $[\alpha]_D^{20}$ −13·2° in pyridine (*c*, 0·53) (Found: C, 67·9; H, 8·8. Calc. for $C_{27}H_{42}O_7$: C, 67·8; H, 8·8%). Direct comparison between IV and synthetic pregnenolone β-D-glucopyranoside (m.m.p., IR), showed them to be identical.

*Methanolysis of permethylates.* Permethylates of I, II and III were refluxed with 5% methanolic HCl for 1·5 hr, neutralized with IRA-410 and MeOH was evaporated *in vacuo*. To the residue, $H_2O$ was added and insoluble substance was filtered (pregnenolone). After evaporation *in vacuo*, the residues were subjected to GLC for methyl sugars. Relative $R_t$ of methyl sugars from I permethylate: 0·77,* 1·0, 4·6, 5·4; from II permethylate: 0·78,* 1·0, 1·7,* 2·3; from III-permethylate: 0·78,* 1·0, 2·0, 2·3,* (Me 2,3,4,6-tetra-*O*-Me-α-D-glucoside: 1·00 ($R_t$: 29·5 min); Me 2,3,4-tri-*O*-Me-α-: 2·3; Me 2,4,6-tri-*O*-Me-α-: 2·8; Me 3,4,6-tri-*O*-Me-α-: 2·0, Me 3,4-di-*O*-Me-α-: 4·6; Me 3,4-di-*O*-Me-β-: 5·4).

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* Minor peak, probably assigned as corresponding Me-β-D-glucoside.

[6] F. A. LOEWUS, *Analyt. Chem.* **24**, 219 (1952).