Phytochemistry, 1973, Vol. 12, pp. 1509 to 1510. Pergamon Press. Printed in England.

3β-HYDROXY-$\Delta^{5,16}$-PREGNADIEN-20-ONE FROM *VERATRUM GRANDIFLORUM*

K. KANEKO, M. WATANABE and HIROSHI MITSUHASHI

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

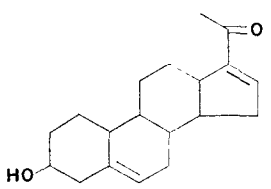
(*Revised Received* 24 *November* 1972. *Accepted* 11 *December* 1972)

Key Word Index—*Veratrum grandiflorum*; Liliaceae; neutral glycoside; steroid; Δ^{16}-pregnenolone.

In our studies on the biosynthesis of *Veratrum* alkaloids[1-3] we have systematically investigated the steroids obtained after enzymic (emulsin) hydrolysis of the neutral glycosidic fraction isolated from budding *Veratrum grandiflorum* (Maxim.) O. Loes. (Liliaceae). In addition to the steroid sapogenin yamogenin ((25*S*)-Δ^5-spirostene-3β-ol), the sterol sitosterol, and the cholesterol derivatives dormantinone ((25*S*)-3β,26-dihydroxy-Δ^5-cholestene-22-one)[4] and dormantinol ((25*S*)-Δ^5-cholestene-3β,22ξ,26-triol),[4] a pregnane derivative has been isolated which is now identified as 3β-hydroxy-$\Delta^{5,16}$-pregnadiene-20-one (I).

RESULTS

Compound I was isolated after silica gel, alumina column chromatography, and had m.p. 208–210° (acetone, plate form). In the Liebermann–Burchard reaction and with $SbCl_3$, it gave reddish-yellow to black, and pink colours, respectively. These results suggest the steroidal structure with Δ^5-3β-ol moiety for (I).



(I) 3β-Hydroxy-$\Delta^{5,16}$-pregnene-20-one

The IR spectrum of (I) showed absorption band at 3400–3450 cm^{-1} for hydroxyl group and strong absorptions at 1660 and 1590 cm^{-1}, characteristic of the α,β-unsaturated ketone group in Δ^{16}-20-one. Its UV max at 239·5 nm (ε 9000) supported a Δ^{16}-20-one structure.[5] The nature of pregnene structure was indicated by its MS, [M^+] 314, *m/e* 299 (M–Me), 296 (M–H_2O), 281 (M–Me–H_2O), 271 (M–MeCO·), 253 (271–H_2O).

[1] KANEKO, K., MITSUHASHI, H., HIRAYAMA, K. and YOSHIDA, N. (1970) *Phytochemistry* **9**, 2489.
[2] KANEKO, K., WATANABE, M., TAIRA, S. and MITSUHASHI, H. (1972) *Phytochemistry* **11**, 3199.
[3] KANEKO, K., WATANABE, M., KAWAKOSHI, U. and MITSUHASHI, H. (1971) *Tetrahedron Letters* 4251.
[4] KANEKO, K., WATANABE, M. and MITSUHASHI, H. (1972) *Abstr. Papers, Annu. Meet. Pharm. Soc. Japan* **II**, 255; details will be published in the following paper.
[5] MARKER, R. E., WAGNER, R. B., ULSHAFER, P. R., WITTBECKER, E. L., GOLDSMITH, D. P. J. and RUOF, C. H. (1947) *J. Am. Chem. Soc.* **69**, 2167; BUTENANDT, A. and SCHMIDT-THOME, J. (1939) *Chem. Ber.* **72**, 182.

From these results, the structure of (I) is proposed as 3β-hydroxy-$\Delta^{5,16}$-pregnadien-20-one, and IR, UV and MS of I were completely identical with those of authentic sample. Also no depression in m.p. was observed on admixture with an authentic specimen.

Schreiber and Aurich,[6] and Heftmann *et al.*[7] previously isolated 3β-hydroxy-Δ^{16}-5α-pregnene-20-one from *Lycopersicon pimpenellifolium* (Solanaceae) and they showed that this Δ^{16}-pregnene is a product of the biological degradation of the steroidal alkaloid glycoside tomatin. Recently, Heftmann *et al* [8] have shown that this Δ^{16}-pregnene is also produced from neotigogenin in same plant. Also, 3β-hydroxy-$\Delta^{5,16}$-pregnadien-20-one glycoside has been isolated from the rhizome of *Paris polyphylla* by Kawasaki *et al.*[9] Thus, 3β-hydroxy-$\Delta^{5,16}$-pregnene-20-one (I) is probably formed from spirostane derivatives, because *Veratrum* and *Paris* contain a considerable amount of spirostane, either yamogenin or diosgenin.

EXPERIMENTAL

The aerial part of budding *Veratrum* was harvested in early April at Teine, Hokkaido, Japan, and 5·34 kg of powder was obtained by extraction with ammoniacal $CHCl_3$–MeOH, and 890·4 g of the extract was separated into 328 g of alkaloidal and 112 g of neutral glycosides by the use of tartaric acid. Neutral glycoside 112 g was hydrolyzed with 5 g of emulsin at pH 5·25 in acetate buffer at 37° for 14 days. The hydrolyzate was extracted with Et_2O. The Et_2O extract (yield, 39 g) was chromatographed over 1200 g of silica gel with C_6H_6 (5 l) and the column successively eluted with 10% Et_2O in C_6H_6 (14 l.), $CHCl_3$ (12 l), and 5% MeOH in $CHCl_3$(12 l.). Sitosterol (VI) and yamogenin (IV) were eluted with 10% Et_2O in C_6H_6 and dormantinone (II) and dormantinol (III) were eluted with 5% MeOH in $CHCl_3$ [4] The $CHCl_3$ fraction (1·2 g) was rechromatographed over 36 g of alumina column with C_6H_6 (1·5 l.) and then with $CHCl_3$ (1 l). The C_6H_6 fraction afforded 10·1 mg of (I), as crystals, m.p. 208–210° (uncorrected), $[\alpha]_D^{20}$ −33·7° (*c* 1, $CHCl_3$).

Acknowledgements—This work was supported in part by funds from the Takeda Chemical Foundation.

[6] SCHREIBER, K. and AURICH, O. (1966) *Phytochemistry* **5**, 707.
[7] BENNETT, R. D., LIEBER, E. R. and HEFTMANN, E. (1967) *Phytochemistry* **6**, 837.
[8] HEFTMANN, E and SCHWIMMER, S. (1972) *Phytochemistry* **11**, 2783.
[9] FUKUDA, R , NOHARA, N. and KAWASAKI, T. (1972) *Abstr. Papers, Annu. Meet Pharma. Soc. Japan* **II**, 249.