earlier [1]. Cleavage of the C-24, C-28 bond gives the fragment ion at *m/e* 409 which does not contain a deuterium label (the intensity of peak at *m/e* 410 is 30% that of *m/e* 409). This result is fully consistent with the presence of one deuterium label at C-28 and the other at C-29 in the oogoniols.

Mass spectral data thus indicates that the primary hydroxyl is located at C-29. We have recently obtained evidence from ^{13}C NMR spectra of oogoniol-1 and model 3β,26- and 3β,29-dihydroxystigmast-5-enes, which also supports the presence of a C-29 hydroxyl in the oogoniols [4].

Biosynthesis from [CD_3]-methionine gives oogoniol with one deuterium label at C-28 and one at C-29 so that two deuterium atoms are lost from C-29 in the conversion of the fucosterol to oogoniol. A plausible way in which this could occur is for fucosterol to be oxidized to an alcohol then to an aldehyde. A similar oxidation takes place in the biosynthesis of antheridiol, for a C-29 carboxylic acid derived from fucosterol has been shown to be an intermediate [5]. However, there is some indication that formation of a double bond at C-22, C-23 precedes oxidation of methyl to carboxyl at C-29. Oogoniol does not possess oxygen functions at C-22 or C-23 so its biosynthesis may not involve such an intermediate. The possibility has been considered that oogoniol may be derived from antheridiol. This does not appear likely because oogoniol possesses a deuterium label at C-29 which would have been lost on oxidation of fucosterol to a C-29 carboxylic acid. If 29-oxo-fucosterol is indeed an intermediate then it may be converted to oogoniol by reduction of the C-24, C-28 double bond, reduction of aldehyde to alcohol, hydroxylation at C-11 and C-15 and oxidation at C-7. We are planning further experiments to define the sequence in which these reactions occur.

EXPERIMENTAL

Feeding experiments were carried out in a similar way to those described previously [2]. Production medium contained appropriate amounts of [CD_3]-methionine as indicated in the text. Steroids were isolated and purified by chromatography before determination of mass spectra. For oxidation of oogoniol-2, the purified sample was treated with Jones' reagent for 1 hr at room temp. H_2O was added and the resulting ppt. extracted with $CHCl_3$ and the extract purified by TLC. The acid was methylated by adding a few drops of ethereal CH_2N_2 and allowing the soln to stand for 15 min before removing excess reagent.

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REFERENCES

1. McMorris, T. C., Seshadri, R., Weihe, G. R., Arsenault, G. P. and Barksdale, A. W. (1975) *J. Am. Chem. Soc.* **97**, 2544.
2. McMorris, T. C. and White, R. H. (1977) *Phytochemistry* **16**, 359.
3. Lederer, E. (1969) *Q. Rev. Chem. Soc.* **23**, 453.
4. McMorris, T. C., Schow, S. R. and Weihe, G. R. (1978) *Tetrahedron Letters* 335.
5. Popplestone, C. R. and Unrau, A. M. (1974) *Can. J. Chem.* **52**, 462.

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ISOLATION OF 3β-HYDROXY-5α-PREGNAN-16-ONE FROM *SOLANUM HAINANENSE*

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Key Word Index—*Solanum hainanense*; Solanaceae; plant steroids; pregnanes; 3β-hydroxy-5α-pregnan-16-one; biosynthesis.

A variety of neutral pregane derivatives occur in higher plants [1–3], while more recently the isolation of pregnane glycosides has been reported [4]. We now wish to report the isolation and structure of another pregnane derivative, isolated from the Vietnamese Solanacea *Solanum hainanense* Hance, which we have identified as 3β-hydroxy-5α-pregnan-16-one.

Al_2O_3 chromatography of the $CHCl_3$ extracts of dried roots yielded 0.025% of the neutral compound $C_{21}H_{34}O_2$ (M^+ *m/e* 318), mp 153°, which showed a deep blue colour upon detection with iodine on the TLC plate. The IR (nujol) spectrum indicated a hydroxyl absorption at 3200 cm^{-1} (broad) and a 5 membered carbonyl function at 1741 cm^{-1}, the latter also established by UV absorption at λ_{max} (ε) 299 (58). The 60 MHz ^1H NMR spectrum was in agreement with a steroidal character giving diagnostic signals at δ 0.64 (s, C-18), 0.78 (s, C-19) and 3.53 ppm (m, 3α-H). The MS showed fragment ion at *m/e* 303 (M^+-CH_3), 300 (M^+-H_2O), 285 (M^+-CH_3—H_2O), the base peak at *m/e* 234 (M^+-84) was due to a loss of ring D by fission between C-13, C-17 and C-14, C-15 as found for other steroidal 16-ketones [5]. The ORD curve gave a strong negative Cotton effect ($a = -180.3$) at 298 nm, typical of a C-16 carbonyl steroid of the 14α-series [6]. These data suggested that the isolated plant steroid was 3β-hydroxy-5α-pregnan-16-one whose structure was finally supported by direct comparison with an authentic sample synthesized as described earlier [7].

The 16-keto pregnane could not be detected in the leaf extracts of *S. hainanense*. The isolation of the new spirosolane alkaloid solasodenone from the $CHCl_3$ extracts of roots and leaves of this plant has been reported recently [8] whereas the glycosidic fractions of the same plant contain solasodine as the main aglycone (unpublished results).

The occurrence of 3β-hydroxy-5α-pregnan-16-one in this plant is of particular interest with regard to its biogenesis. All the other neutral pregnanes isolated from higher plants show an oxygen function at C-20

and are assumed to be produced via 3β-hydroxy-pregn-5-en-20-one or progesterone derived from cholesterol or sitosterol [2]. Some Δ^{16}-20-keto-pregnanes could be assumed to be formed in plants from co-occurring spirostanes [3, 4, 9] or furostane derivatives as well as spirosolane alkaloids [10, 11] in a manner similar to the corresponding Marker type chemical degradation. It is unknown if 3β-hydroxy-5α-pregnan-16-one in *S.hainanense* is produced as a secondary product of one of the above degradation pathways or by a new biosynthetic route. However, the present findings show that the possibilities for pregnane biosynthesis in higher plants seem to be more various than assumed up to now [12].

EXPERIMENTAL

UV spectra were measured in MeOH; ORD determinations were in MeOH; NMR spectra were taken in $CDCl_3$ with HMDS as internal standard.

Isolation of 3β-hydroxy-5α-pregnan-16-one. Dried and powdered roots (100 g), collected near Hanoi, Vietnam, were extracted exhaustively with $CHCl_3$ in a Soxhlet apparatus, $CHCl_3$ was concd to 1/3 and extracted 3 × with petrol to remove pigments and lipids. Evapn of the $CHCl_3$ gave a residue which was chromatographed over Al_2O_3 (Woelm, neutral, grade I). The progress of the separation was followed by TLC on Si gel ($CHCl_3$–EtOH, 9:1). Elution with $CHCl_3$–EtOH (8:2) yielded 3β-hydroxy-5α-pregnan-16-one. Needles (Me_2CO–H_2O), mp 153°, $[\alpha]_D^{26}$ − 47.3° (c = 0.300, in EtOH), R_f 0.74, identical mmp, R_f, IR, ORD) with an authentic specimen [7].

Further elution with $CHCl_3$–EtOH (7:3) gave solasodenone as already described [8].

REFERENCES

1. Heftmann, E. (1970) in *Steroid Biochemistry* p. 75. Academic Press, New York.
2. Heftmann, E. (1973) in *Phytochemistry* (Miller, L. P. ed.), p. 198. van Nostrand-Reinhold, New York.
3. Kiyosawa, S. and Kawasaki, T. (1977) *Chem. Pharm. Bull.* **25**, 163.
4. Kiyosawa, S., Goto, K., Owashi, R. and Kawasaki, T. (1977) *Tetrahedron Letters* No. 52, 4599.
5. Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1964) in *Structure Elucidation of Natural Products by Mass Spectrometry* Vol. 2, p. 19, Holden-Day, San Francisco.
6. Shoppee, C. W. (1964) in *Chemistry of the Steroids* (2nd edn) p. 35, Butterworths, London.
7. Adam, G. and Schreiber, K. (1967) *Liebigs Ann. Chem.* **709**, 191.
8. Adam, G., Lischewski, M., Huong, H. Th. and Khoi, N. H. (1978) *Phytochemistry* **17**, 1070.
9. Heftmann, E. (1975) *Phytochemistry* **14**, 891.
10. Heftmann, E. and Schwimmer, S. (1974) *Phytochemistry* **11**, 2783.
11. Schreiber, K. (1968) in *The Alkaloids—Chemistry and Physiology*, (Manske, R. H. F., ed.), Vol 10, p. 38. Academic Press.
12. Geuns, J. M. C. (1978) *Phytochemistry* **17**, 1.

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A NATURAL APOCAROTENOL FROM THE PEEL OF THE RIPE GOLDEN DELICIOUS APPLE

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Key Word Index—Golden Delicious apple; natural apocarotenols.

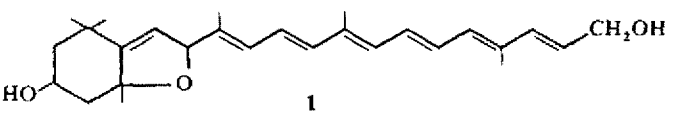
INTRODUCTION

In various fruits, polar UV fluorescent pigments with sharp fine structure, typical of short in-chain chromophores, were found. They were named according to their source, valenciaxanthin, valenciachrome, from Valencia and Navel orange peel [1, 2]. Persicaxanthin and persicachrome were detected in Rosaceae (peaches, apricots, prunes) [3, 5] and in other fruits with low carotenoid content (figs, blackberries, grapes) [6]. Their structure is still unknown [7].

In the pulp of avocado fruits, *Persea americana*, two new UV fluorescent apocarotenoids were identified by Gross *et al.* [8]. One of these was assigned the structure 5,8-epoxy-5,8-dihydro-10'-apo-β-carotene-3,10'-diol (1):

The pigment is related to the natural apo-10'-violaxanthal (5,6-epoxy-3-hydroxy-5,6-dihydro-10'-apo-β-caroten-10'-al) found in Valencia orange peels [9]. This compound (λ_{max} 440 nm) was reduced to the corresponding alcohol λ_{max} 370, 392, 414 nm. On treatment with HCl the maxima shifted to 352, 371, 394 nm, through 5,8-isomerization of the 5,6-epoxide. Curls *in vitro* product appears to be identical with the apocarotenol (1) found in avocado.

While investigating the carotenoid changes during the ripening of Golden Delicious apples, we detected a similar pigment that appeared only in ripe fruit, and its structure was investigated.



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