fragments usually observed at *m/z* 99 and 81 due to cleavage at C-20, C-22 were not detected.

Since **2** on weak base treatment regenerated the acetyl-free compound **1**, whose R_f value on TLC was coincident with that of the compound in the original methanolic extractive, it was considered that **1** probably exists in an acetyl-free form in the plant material.

Moulting test of **1**. A suspension of **1** in H_2O (2 mg/ml) was used as a test sample. An aq. soln of **3** (2 mg/ml, Rohto Pharmaceutical Co. Ltd.) for reference and H_2O for control were used. Each soln was injected directly into the centre of the abdomen or administered orally into the alimentary canal, using a micro-syringe at a dose of 10 μg (0.005 ml)/larva (5th instar), respectively.

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TRACE FORMATION OF PROGESTERONE FROM 22-DEHYDRO PHYTOSTEROLS DURING STORAGE

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Key Word Index—Progesterone; 22-dehydro phytosterols; side chain oxidation; storage.

Abstract—Progesterone has been isolated from different samples of 22-dehydro phytosterols of previously known composition, kept in a laboratory collection for periods from 10 to 18 years. It is not present in detectable amount in cholesterol, fucosterol or desmosterol, or in freshly prepared samples of stigmasterol. The possibility of progesterone being an autoxidation artifact is thus emphasized, in particular in the environment, and hence, in concentrating organisms.

During the course of recrystallization of sterol samples of previously known composition which had been kept in the laboratory collection for several years, we observed strongly UV absorbing substances accumulating in the mother liquors. A substance having the same R_f as progesterone was detected in the case of old samples of 22-dehydro sterols while the corresponding spot was absent in samples of other sterols. A progesterone fraction was isolated from an 18 years old sample of stigmasterol of known composition (checked by MS, TLC, GC) after two successive chromatographies of the methanol mother liquors on Si gel. This product had the expected molecular ion for progesterone at *m/z* 314 with the characteristic [1] fragments at *m/z* 43 $[MeCO]^+$, 272 ($[M-42]^+$, elimination of ketene from the 3-oxo 4-ene conjugated system) and 299 $[M-15]^+$. A second molecular ion at *m/z* 316 of nearly equal intensity indicated a derivative which, due to scarcity of the isolated fraction, could not be separated. The UV spectrum (240 nm, ethanol) was in agreement with an α, β-unsaturated carbonyl group. A 2, 4-dinitrophenylhydrazone was prepared from a methanolic solution and submitted to re-isolation by TLC. The product showed the same R_f as an authentic sample of progesterone-2, 4-DNP, and gave a mass spectrum with the molecular ion at *m/z* 674 for a bis-2, 4-DNP.

A systematic investigation was performed on the other sterol samples in the collection, and progesterone was found in all the aged 22-dehydro phytosterol-containing products. Progesterone was also isolated from several pollen sterols of reported composition [2], together with a series of UV absorbing products which have not been further investigated due to scarcity. Thus, when originally isolated in 1967 [2] from a mustard pollen (*Brassica nigra*), a sterol fraction contained 37% methylene cholesterol, 9% mono-unsaturated C_{27} sterol, 32% di-unsaturated C_{29} sterol and 7% mono-unsaturated C_{29} sterol. After recrystallization in methanol, the relative concentrations have now changed to 10% for the C_{28} and

11% for the C_{29} di-unsaturated sterols according to mass spectrometry.

Progesterone was not found in aged samples of cholesterol, sitosterol, fucosterol and desmosterol when using the same methods. It is also absent in freshly prepared samples of stigmasterol.

The air oxidation of cholesterol is known [3] to produce *ca* 20 C_{27} derivatives, among which the 7-keto and 25-hydroxy compounds have been identified. Desmosterol is highly sensitive to oxidation even in the solid state, and gives at least 10 products [4] which have not yet been fully investigated to the author's knowledge. Fucosterol on storage was shown [5] to oxidize mainly to 24-ketocholesterol and saringosterol, but minor components present in the mixture have not been isolated.

The formation of 3-oxo components by oxidation of cholesterol in the solid state under the action of molecular oxygen has been reported recently [6] but the products thus formed have not been identified.

The oxidative cleavage of the 22-dehydro phytosterols with the elimination of the side chain carbons is certainly a slow process when occurring in the crystalline state but it is possibly more important under natural conditions. A probable mechanism of progesterone formation may proceed through: (1) peroxidation of the Δ^{22} bond of the side chain; (2) intermediate production of a $\Delta^{20(22)}$ unsaturation; and (3) oxidation of this new double bond leading to the methyl ketone at C-17.

The occurrence of progesterone among invertebrates (for reviews see Refs. [7, 8]) suggests the need to demonstrate the *in vivo* biosynthesis of this steroid from labelled precursors, as such animals may concentrate it from the environment.

EXPERIMENTAL

TLC (Si gel, hexane–EtOAc, 2:1) was performed on the methanolic mother liquors obtained from the sterol crystallizations. Spots were detected by UV observation. Progesterone (R_f 0.40) was isolated from the TLC band by Et_2O elution and the 2,4-dinitrophenylhydrazone obtained by adding a few drops of 2,4-DNPH-HCl in MeOH. Prep. TLC afforded the progesterone-2,4-DNP (R_f 0.55). In the case of an 18 years old sample of stigmasterol, 202 mg sterol gave after crystallization 93 mg of a yellowish, MeOH-soluble fraction, leading to 1.4 mg of progesterone fraction after two prep. TLC separations.

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