THE METABOLIC ROLE OF THE 24-ETHYLIDENECHOLESTEROLS*

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Several epimeric 24-ethylidenecholesterols have been isolated from certain species of plants (Heilbron, Phipers and Wright (1934); Tsuda, Hayatsu, Kishida and Akagi (1958); Knights (1965); Baisted (1967)). The manner in which they arise has been postulated (Castle, Blondin and Nes (1963); Birch (1963); Bader, Guglielmetti and Arigoni (1964); Hügel, Vetter, Audier, Barbier, and Lederer (1964); Lederer (1964)) to involve the addition of the methyl group of methionine to a 24-methylenesterol. In various species of brown algae it is already known that fucosterol does indeed arise via mevalonate with the addition of two methyl groups from methionine (Villanueva, Barbier and Lederer (1964, 1965); Mercer (1965); Goad, Hammam, Dennis and Goodwin (1966); Castle, Blondin and Nes (in press)). 24-Ethylidenesterols could however also give rise to the 24-ethylsterols by reduction with pyridine nucleotide in a manner similar to the known reduction of the Δ^{24}-bond (Stokes, Hickey and Fish (1958); Steinberg and Avigan (1960); Avigan and Steinberg (1961)). If this were true, the 24-ethylidenesterols should be present quite generally in plants which form the 24-ethyl compounds. We would like to report here the isolation of both 29-isofucosterol and β-sitosterol from Pinus pinea, the biosynthesis of

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29-isofucosterol from mevalonate in this species, its formation from 24-methylenecholesterol, and its conversion to 24-ethylcholesterol presumably by reduction. 24-Methylenecholesterol similarly was converted to 24-methylcholesterol. The data obtained are consistent with the pathway: Δ^{24} to 24-methylene to 24-ethylidene to 24-ethyl and with the conversion: 24-methylene to 24-methyl (see also Castle, Blondin and Nes (1963) and Lederer (1967) for alternatives). Fucus species (which accumulate the 24-ethylidenesterol) thus appear to be in a category with the yeasts (which accumulate $\Delta^{5,7}$-sterol) both of which have a genetic block at a reductive step. In most organisms investigated, the $\Delta^{24(28)}$- and Δ^{7}-bonds are found reduced in the dominant sterol.

From the ungerminated seeds of Pinus pinea there was obtained by the usual extraction and chromatographic procedures colorless plates, m.p. 137-140°, $[\alpha]_D^{24}$-33.2° ($CHCl_3$), infrared and n.m.r. spectra identical with β-sitosterol. Gas-liquid chromatography showed the sample also contained 30% of 24-methylcholesterol. From thin layer chromatography on silver nitrate impregnated silica gel of the crude acetylated sterol mixture 29-isofucosterol acetate was isolated as a very minor constituent, 8% of total sterol, 6.0 mg. from 350 seeds, m.p. 127-130°, m.p. of the free alcohol 129-131°, λ_{max} 12.25 μ which distinguishes 29-isofucosterol from fucosterol (Dusza (1960)), thin layer (as the acetate, $AgNO_3$-impregnated) and g.l.c. (silanized, XE-60) movements identical with synthetic 29-isofucosterol. Authentic fucosterol (from Fucus) moved slightly faster than its 29-isomer (synthetic) in both the latter procedures.

Incubation of the seeds (lacking their outer coat) with 2-C^{14}-mevalonate yielded a radioactive substance chromatographing as the acetate on a $AgNO_3$-thin layer of silica gel precisely as did an authentic sample of the acetate of 29-isofucosterol. Ozonization yielded 24-ketocholesterol acetate without loss of radioactivity. The ketone was identified by m.p. (126.5-127.5°), I.R., movement on a thin layer of silica gel, and g.l.c. Similar incubation of synthetic 28-C^{14}-24-methylenecholesterol (in Tween 20) with the seeds yielded 29-isofucosterol identified by $AgNO_3$-thin layer (as the acetate) and gas-liquid

chromatography. Reincubation of the labelled and purified 29-isofucosterol gave β-sitosterol in 3.6% yield, most of the remaining radioactivity being recovered as unchanged substrate. The labelled β-sitosterol chromatographed (as the acetate on $AgNO_3$-TLC) as did an authentic sample and when converted to the 3,5-cycloderivative failed to undergo a reaction with osmium tetroxide. By contrast, labelled 29-isofucosterol was converted to a chromatographically highly polar substance by this technique as is labelled 24-methylenecholesterol (Russell, van Aller and Nes (in press)). From the incubation of 28-C^{14}-24-methylenecholesterol in addition to 29-isofucosterol labelled 24-methylcholesterol was obtained, identified by gas-liquid chromatography of the side chain saturated portion of the $AgNO_3$-thin layer chromatogram.

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